Medical–Biological
Research & Technologies

# BioMagPure Extraction Kit Handbook

## For automated purification of nucleic acids using the BioMagPure System

BS-060201-AK BioMagPure Blood DNA Extraction Kit 200
BS-060201-BK BioMagPure Blood DNA Extraction Kit 1200
BS-060201-CK BioMagPure Viral Nucleic Acid Extraction Kit
BS-060201-DK BioMagPure Tissue DNA Extraction Kit
BS-060201-EK BioMagPure Cultured Cell DNA Extraction Kit
BS-060201-FK BioMagPure Bacterial DNA Extraction Kit
BS-060201-GK BioMagPure HPV DNA Extraction Kit for Swab Samples
BS-060201-IK BioMagPure TB DNA Extraction Kit
BS-060201-JK BioMagPure FFPE DNA Extraction Kit
BS-060201-KK BioMagPure Forensic DNA Extraction Kit
BS-060201-LK BioMagPure Viral/Pathogen Nucleic Acids Extraction Kit A
BS-060201-MK BioMagPure Viral/Pathogen Nucleic Acids Extraction Kit B
BS-060201-NK BioMagPure Viral RNA Extraction kit
BS-060201-OK BioMagPure Plant DNA Extraction Kit
BS-060201-PK BioMagPure Total RNA Extraction Kit
BS-060201-QK BioMagPure Viral Nucleic Acid Large Volume Extraction Kit
BS-060201-RK BioMagPure CFC DNA Extraction Kit LV

For in vitro diagnostic use
Remarks: Plant DNA Extraction Kit and Total RNA Extraction Kit are not classified as CE IVD products.

Version: 5.6
Mod. date: 01/10/15
Manual № Z3001

CE IVD EC REP H B

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Whole blood<br>Fresh, frozen, anticoagulated | gDNA | ● | ● | | | | | | | | △ | | | | | | | |
| | Bacteria | | | | | | ● | | | | | | | | | | | |
| | Mycobacteria | | | | | | | | ● | | | | | | | | | |
| | Total RNA | | | | | | | | | | | | | | | ● | | |
| Buffy coat | gDNA | ● | ● | | | △ | | | | | | | | | | | | |
| | Total RNA | | | | | | | | | | | | | | | ● | | |
| Leukocyte concentration | gDNA | ● | ● | | | △ | | | | | | | | | | | | |
| | Total RNA | | | | | | | | | | | | | | | ● | | |
| Clotted, dried blood | gDNA | | | | △ | | | | | | ● | | | | | | | |
| Plasma and serum | Viral DNA/RNA, circulating DNA | | | ● | | | | | | | | △ | | | | | ● | |
| | Viral DNA | | | △ | | | | | | | | △ | | ● | | | △ | |
| | Virus + bacteria | | | | | | | | | | | ● | | | | | | |
| | Bacteria | | | | | | ● | | | | | △ | | | | | | |
| | Mycobacteria | | | | | | | | ● | | | | | | | | | |
| Blood stain | gDNA | | | | ● | | | | | | △ | | | | | | | |
| Bone marrow | gDNA | | | | ● | △ | | | | | △ | | | | | | | |
| | Total RNA | | | | | | | | | | | | | | | ● | | |
| CFS, urine, other cell-free bodily fluids | Viral DNA/RNA, circulating DNA | | | ● | | | | | | | | △ | | | | | ● | |
| | Viral RNA | | | △ | | | | | | | | △ | | ● | | | △ | |
| | Virus + bacteria | | | | | | | | | | | ● | ● | | | | | |
| | Bacteria | | | | | | ● | | | | | △ | | | | | | |
| | Mycobacteria | | | | | | | | ● | | | | | | | | | |
| Cell culture supernatant | Viral DNA/RNA | | | ● | | | | | | | | △ | | | | | ● | |
| | Viral RNA | | | △ | | | | | | | | △ | | ● | | | △ | |
| | Bacteria + Virus | | | | | | | | | | | ● | ● | | | | | |
| | Bacteria | | | | | | ● | | | | | △ | | | | | | |
| Bronchoalveolar lavage, aspirates | gDNA | | | | | ● | | | | | | | | | | | | |
| | Bacteria | | | | | | ● | | | | | | | | | | | |
| | Bacteria + virus | | | | | | | | | | | | ● | | | | | |
| | Mycobacteria | | | | | | | | ● | | | | | | | | | |
| Liquid sample transport media | Viral DNA/RNA | | | ● | | | | | | | | △ | | | | | ● | |
| | Viral RNA | | | △ | | | | | | | | △ | | ● | | | △ | |
| | HPV virus | | | | | | | ● | | | | | | | | | | |
| | Bacteria | | | | | | ● | | | | | △ | | | | | | |
| | Virus + bacteria | | | | | | | | | | | | ● | | | | | |
| Bacterial culture: suspension, plate, colony | Bacteria (gram+ and gram-) | | | | | | ● | | | | | | ● | | | | | |
| | Mycobacteria | | | | | | | | ● | | | | | | | | | |
| Sputum, decontaminated sputum | Bacteria | | | | | | ● | | | | | | | | | | | |
| | Mycobacteria | | | | | | | | ● | | | | | | | | | |
| | Bacteria + virus | | | | | | | | | | | | ● | | | | | |
| Saliva, mouthwash | gDNA | | | | | | | | | | ● | | | | | | | |
| | Bacteria | | | | | | ● | | | | | | | | | | | |
| | Bacteria + virus | | | | | ● | | | | | | | ● | | | | | |

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cultured cells | gDNA | | | | | | | | | | | | | | | ● | | |
| | Total RNA | | | | ● | | | | | | | | | | | | | |
| Solid tissue, biopsy | gDNA | | | | | | | | | | △ | | | | | ● | | |
| | Total RNA | | | | | | ● | | | | | | | | | | | |
| | Bacteria | | | | ● | | | | | | | | | | | | | |
| Rodent tails | gDNA | | | | | | | | | | | | | | | ● | | |
| | Total RNA | | | | | | | | | ● | | | | | | | | |
| FFPE tissue | gDNA | | | | | | | ● | | | | | | | | | | |
| Cervicovaginal sample: scrapes, smear (brush, swab), lavage | HPV virus | | | | | | ● | | | | | | △ | | | | | |
| | Bacteria | | | | | | | | | | | | ● | | | | | |
| | Bacteria + virus | | | | ● | | | | | | △ | | | | | | | |
| Swabs: nasal, pharyngeal, throat, eye, buccal, rectal, genital, skin lesions and mucous membranes contact swabs | gDNA | | | | | | ● | | | | | | △ | | | | | |
| | Bacteria | | | | | | | | | | | | ● | | | | | |
| | Bacteria + virus | | | | | | | | | | | | ● | | | | | |
| | Virus | | | | | | ● | | | | | | ● | | | | | |
| Stool | Bacteria | | | | | | | | | | | | ● | | | | | |
| | Bacteria + virus | | | | | | | | | | ● | | | | | | | |
| Forensic and human identity samples | gDNA | | | | | | | | | | ● | | | | | | | |
| Chewing gum | gDNA | | | | | | | | | | ● | | | | | | | |
| Cigarette butts | gDNA | | | | | | | | | | ● | | | | | | | |
| Sperm stain | gDNA | | | | | | | | | | ● | | | | | | | |
| Stamps, envelopes | gDNA | | | | | | | | | | ● | | | | | | | |
| Fingernails | gDNA | | | | | | | | | | ● | | | | | | | |
| Hair, hair roots | gDNA | | | | ● | | | | | | | | | | | | | |
| Insects, fish, shrimps | gDNA | | | | | | | | | | | | | | | ● | | |
| | Total RNA | | | | | | | | | | | | | | ● | | | |
| Plant material, GMO, fungi | gDNA | | | | | | | | | | | | | | | ● | | |
| | Total RNA | | | | | | | | | | | | | | ● | | | |
| Yeast | gDNA | | | | | | | | | | | | | | | ● | | |
| | Total RNA | | | | | | | | | | | | | | | | | ● |
| Protein (His-tag) | His-tagged protein (expression system) | | | | | | | | | | | | | | | | | |
| Environmental sample, water | Bacteria | | | | | | ● | | | | | | ● | | | | | |
| | Bacteria + virus | | | | | | | | | | | | ● | | | | | |

Recommended kit: ●
Compatible kit: △

# 2. Introduction

## 2.1. The Biosan Nucleic Acid Purification Technology.

Biosan is specialized in developing advanced, efficient and reliable technologies in nucleic acid purification, enabling successful delivery of extraction results from varied sample types.

The BioMagPure technology is a state of the art platform that uses magnetic bead to extract nucleic acids from samples. The platform commits a truly walk-away automation in nucleic acid purification from samples to results. The purification process contains steps of lysis, binding, washing and elution as figure below.

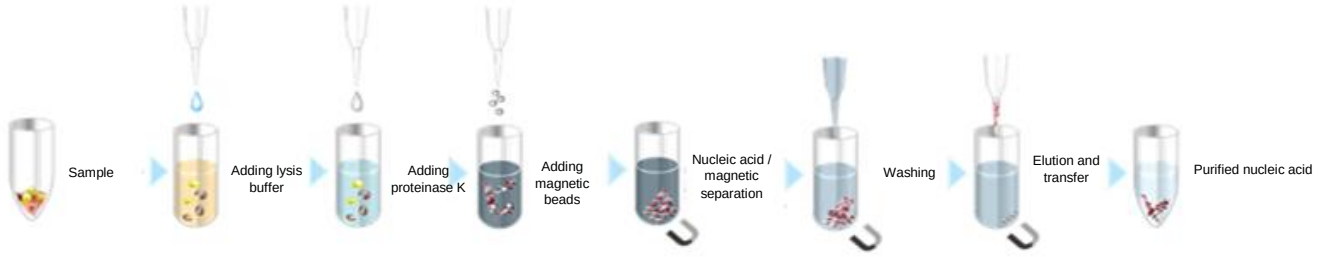



**Figure: BioMagPure magnetic bead extraction process**

# 3. Product information

### 3.1. Intended use

BioMagPure Kits is intended for the purification of nucleic acid from biological specimen used with BioMagPure instruments as IVD accessory.

The nucleic acids purified using the BioMagPure instruments and reagent kits are suitable for a variety of polymerase chain reaction (PCR) tests for human in vitro diagnostics purpose.

The BioMagPure instrument and reagent kits are intended for professional use only.

### 3.2. Product use limitations

The BioMagPure instrument and BioMagPure kit are not intended for use as part of a specific in vitro diagnostic test. The user is responsible for establishing performance characteristics necessary for downstream diagnostic applications. Appropriate controls must be included in any downstream diagnostic applications using nucleic acid purified using the BioMagPure instrument and BioMagPure reagent kits.

### 3.3. Warranty

Biosan is committed to providing our customers with high-quality products and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you have any questions or concerns about our product or service, contact our Technical Support Representatives.

Biosan guarantees the performance of all products according to specifications stated on our product literature. The purchaser/user must determine the suitability of the product for its particular use. We reserve the right to change, alter, or modify any product to enhance its performance and design.

This warranty limits Biosan Corporation’s liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions.

### 3.4. Satisfaction guarantee

For any product that fails to perform satisfactorily due to any reason other than misuse, Biosan will replace it free of charge. Simply call your distributor to get a replacement.

### 3.5. Technical support

For technical assistance and more information, please visit our website at www.Biosan.com or call one of the Biosan Technical Service Departments or local distributors.

### 3.6. Safety information

When working with chemicals or samples, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). You can find, download, view, and print them from our website www.Biosan.com

### 3.7. Manufacturer information

Biosan SIA
Ratsupites 7, build.2, Riga, LV-1067, Latvia
Phone: +371 67426137 Fax: +371 67428101
http://www.biosan.lv

# 4. BioMagPure Blood DNA Extraction Kit 200

Cat. No. BS-060201-AK
Process time: BioMagPure 12S – 50-60 minutes
BioMagPure 24 – 50-70 minutes

### 4.1. Intended use

BioMagPure Blood DNA Extraction Kit is used with the BioMagPure instrument for extraction of DNA from 10-400 µl mammalian whole blood, suspension of mammalian blood cells.

### 4.2. Application

Nucleic acids extracted from BioMagPure Blood DNA Extraction kit can be used in a number of downstream application including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, Southern Blot Analysis

### 4.3. Number of tests

48 extractions

### 4.4. Kit components

| Kit Contents | BS-060201-AK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter Tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 4.5. Reagent cartridge contents

**Cell 1 Cell 2 Cell 3 Cell 4 Cell 5 Cell 6 Cell 7 Cell 8 Cell 9 Cell 10**

| | | |
|---|---|---|
| Cell-1 | Proteinase K solution | 40 µl |
| Cell-2 | Lysis Buffer 2 | 1000 µl |
| Cell-3 | Binding Buffer 1 | 600 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 1 | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | Elution Buffer 1 | 1000 µl |
| Cell-9 | Elution Buffer 2 | 1000 µl |
| Cell-10 | Empty | |

### 4.6. Storage

BioMagPure Blood DNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition.

Store the purified total nucleic acid at 4 °C (short-term, less than 10 days) or aliquot and store at –70°C (long-term) before perform the downstream analysis.

### 4.7. Starting material

| | |
|---|---|
| Sample type | Mammalian Whole blood, Buffy coat, Leukocyte concentration* |
| Target nucleic acid | Total DNA (Genomic DNA, mitochondrial and/or viral DNA)** |
| Sample volume | 100-400 µl whole blood (WBC count less than 2 x $10^4$ cells/ µl); |
| | 100-400 µl leukocyte concentration (contains no more than 5 x $10^6$ cells); |
| | 100-400 µl buffy coat ** |
| Controls / optional internal control *** | Add controls /internal control in the extraction procedure if the downstream analysis needed |
| Elute volume | 50-300 µl |

If the sample volume is less, add the appropriate volume of PBS.

BioMagPure Blood DNA Extraction Kit has proven to work for fresh or frozen blood samples collected in tubes containing common anti-coagulants like EDTA, heparin**** and citrate.

Using fresh whole blood sample (within 1 week, stored at 4-8℃) for extraction is recommended, the total nucleic acid yield and quality would be decreased by time. For longer storage time, whole blood should be frozen and avoid freeze-thaw

This protocol were established for isolating DNA form whole blood of healthy individual, the unhealthy or drug-treated individual (e.g. patients of leukaemia or infection disease) may show abnormal blood quality that may influence the nucleic acid extraction procedure

Using the concentrated buffy coat (purified and free of blood cells), the BioMagPure Culture Cell DNA extraction kit (BS-060201-EK) is recommended

If the whole blood sample is granulocyte-rich (white blood cell no. more than 2 X $10^4$ cells/ µl), dilute blood sample or extract the DNA by the BioMagPure blood DNA extraction kit 1200 (BS-060201-BK) is recommended

Storage of nucleic acid: For short-term storage (up to 10 days), store the tubes at 2–8°C. However, for applications requiring maximum fragment size, such as southern blotting, storage purified DNA at 2–8°C for up to 3 days, as low levels of DNA degradation will occur after this time. For long-term storage, store the tubes at–70°C.

In fact, the extracted product contains total nucleic acid (DNA and RNA), the RNA is not the major product in this kit (about 10%) and would be degraded soon. If the RNA-free product is needed, add some RNase to the

### 4.8. Result

#### 4.8.1. Expected purity and yield

DNA was purified by using the BioMagPure Blood DNA extraction Kit 200 and the BioMagPure Purification Instrument using five different human whole blood (in EDTA-K2 collection tube). The DNA concentration was measured using a NanoDrop® 2000 spectrophotometer. The range of DNA yield is 2-18 µg of the BioMagPure Blood Extraction Kit 200 (from the blood sample of WBCs counts: 2-20x$10^3$ cells/ µl). The DNA yield from whole blood depended on the specific blood donor and blood cell count. The yield from cultured cell depended on the type of cell line due to the variable degree of aneuploidy.

| Sample material | Volume/amount | DNA Yield | Purity |
|---|---|---|---|
| Whole blood (WBC count 1.8 x $10^3$ /ml) | 100 µl<br>200 µl<br>300 µl<br>400 µl | 1-1.2 µg<br>2-2.1 µg<br>2.8-3.1 µg<br>4-4.3 µg | OD260/OD280 >=1.8<br>OD260/OD230 >=1.5 |
| Whole blood (WBC count 4 x $10^3$ / µl) | 100 µl<br>200 µl<br>300 µl<br>400 µl | 1.6-2.1 µg<br>3.8-3.9 µg<br>5.1-5.2 µg<br>8.5-8.8 µg | |
| Whole blood (WBC count 6.9 x $10^3$ / µl) | 100 µl<br>200 µl<br>300 µl<br>400 µl | 2.9-3.1 µg<br>5.8-6.2 µg<br>8.2-8.8 µg<br>11.9-12.5 µg | |
| Whole blood (WBC count 10.9 x $10^3$ / µl) | 100 µl<br>200 µl<br>300 µl<br>400 µl | 4.3-4.6 µg<br>8.7-9.1 µg<br>11.5-12.2 µg<br>16.6-17.5 µg | |
| K562 cells | 6 x $10^5$ cells<br>2 x $10^6$ cells | 9-9.6 µg<br>22-25 µg | |

* For those samples which have low leukocyte count(less than 1 x $10^3$ cells/ µl), concentrating the blood cells by centrifuge at 3000 RPM for 15 min under 4°C and taking the leukocyte concentration for DNA extraction is recommended

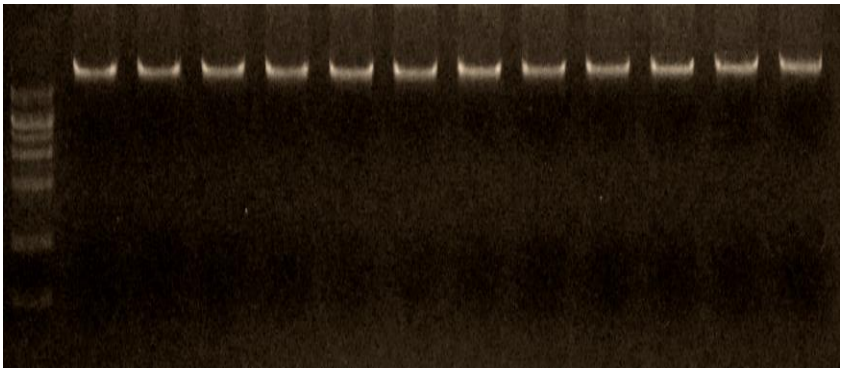
** If the WBC count of the blood sample is more than 2 x $10^4$ cells/ µl, we recommend using BioMagPure Blood DNA Extraction Kit 1200 or diluting the blood sample with PBS (e.g. a whole blood sample from lymphoma/myeloma patient or other granulocyte-rich blood sample / buffy coat)

*** See Controls / internal control below

**** The EDTA is recommended to use as anticoagulation agent, while heparin have inhibit effects on nucleic acid amplification reaction

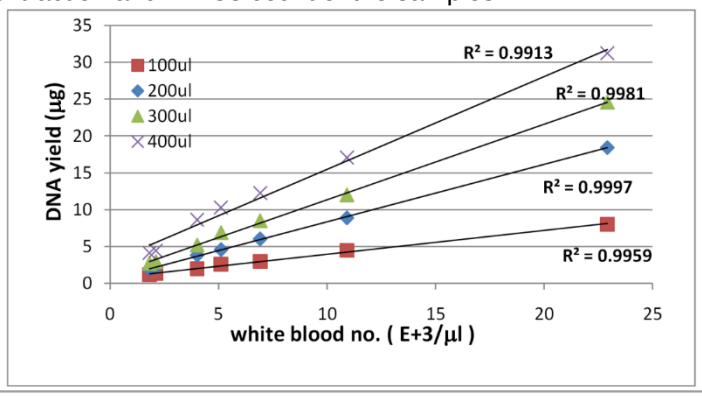
### 4.9. Integrity

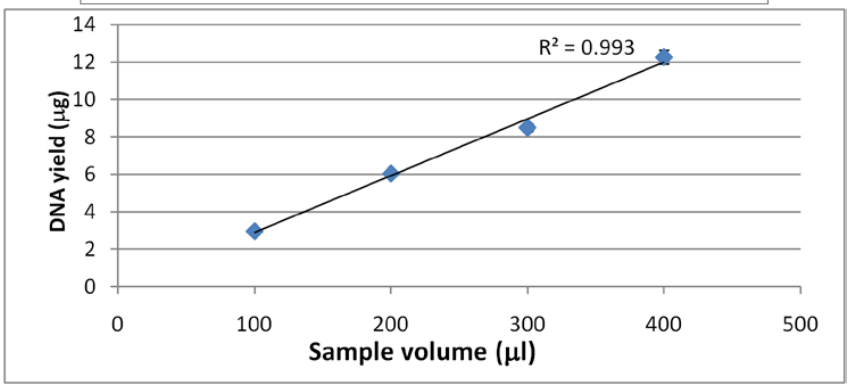
The DNA was isolated in replicates of 12 from 200 µl human whole blood. Elution volume was set to 100 µl. Integrity of DNA was shown by subjecting each eluate to TAE agarose gel electrophoresis together with a Lambda/Hind III (of fragments with size: 0.56, 2.02, 2.32, 4.36, 6.56, 9.42, 23.13 kb). All samples shown single band with molecular weight more than 22kb with no smear.



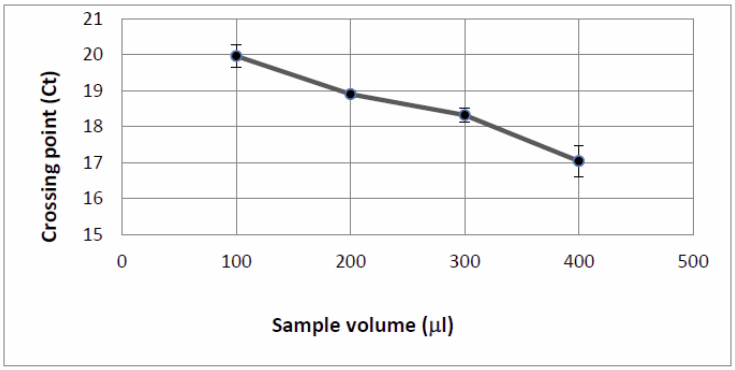
### 4.10. Scalability

The DNA was isolated from different whole blood samples (WBC count range 1.8-22 x $10^3$ cells/ µl). The DNA yield (measured by Nanodrop 2000 UV-Vis spectrophotometer) shows excellent scalability in different volume (100, 200, 300 and 400 µl) extraction and WBCs count of the samples.



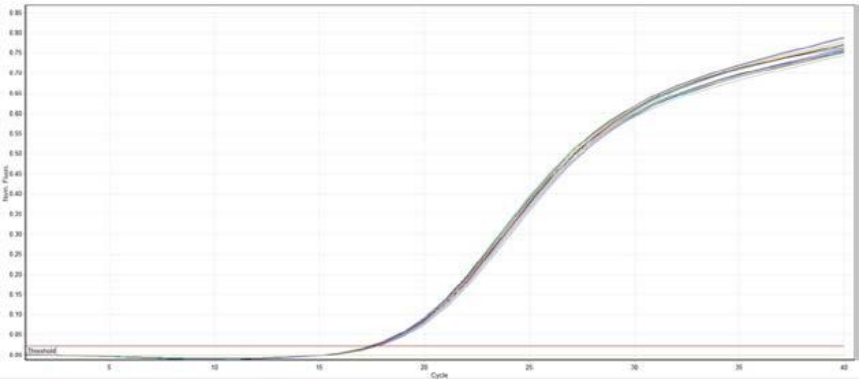




DNA isolated from different amounts of whole blood samples was amplified by real-time qPCR by using the β-globin gene specific primers. The resulting crossing points confirm the scalability on extraction.



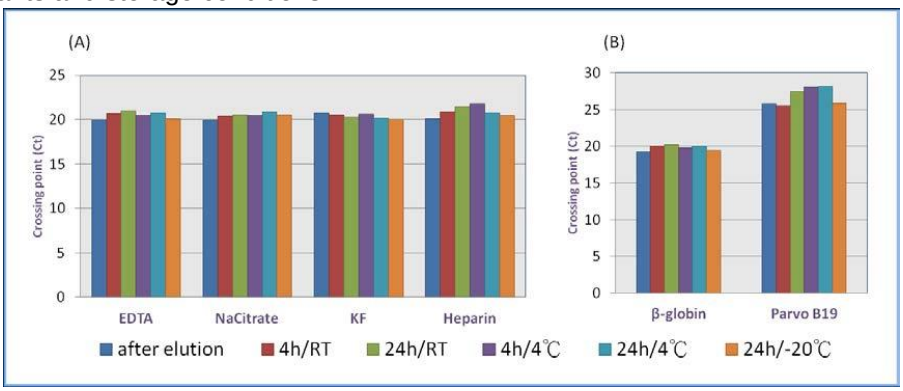
### 4.11. Reproducibility

The DNA were isolated by BioMagPure blood DNA extraction kit from twenty whole blood samples. The β-globin gene was detected by real-time qPCR. This data shows ultra-high stability and reproducibility of BioMagPure 12s nucleic acid purification system.



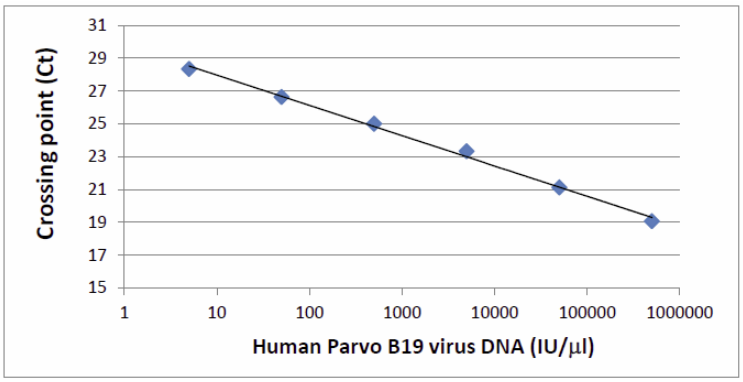
### 4.12. Stability

The DNA were extracted from whole blood by BioMagPure Blood DNA Extraction kit 200. Real-time qPCR detection of the β-globin (A) and spiked parvovirus B-19 DNA (A, B). No significant influences on samples with different anticoagulants and storage conditions.




### 4.13. Sensitivity

Using whole blood (in EDTA collection tube) spiked with serial-diluted human Parvo B19 Virus (in range of 25-2500000 IU/ml). 200 µl sample were extracted and eluted in 100 µl. 10 µl eluate was used for real-time PCR reaction by RealStar® Parvovirus B19 PCR kit 1.0. As little of 5 IU spiked (about 1 IU in PCR reaction) sample can be detected, proving the excellent sensitivity and linearity of isolation procedure.




### 4.14. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 4.15. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Blood DNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

# 5. BioMagPure Blood DNA Extraction Kit 1200

Cat. No. BS-060201-BK
Process time: BioMagPure 12S – 70-100 minutes
BioMagPure 24 – 70-120 minutes

### 5.1. Intended use

BioMagPure Blood DNA Extraction Kit is used with the BioMagPure instruments for extraction of gDNA from 400-1000 µl mammalian blood, suspension of mammalian blood cells.

### 5.2. Application

Nucleic acids extracted from BioMagPure Blood DNA Extraction kit can be used in a number of downstream applications including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, and Southern Blot Analysis.

### 5.3. Number of tests

48 extractions

### 5.4. Kit components

| Kit contents | BS-060201-BK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 5.5. Reagent cartridge contents

**Cell 1** **Cell 2** **Cell 3** **Cell 4** **Cell 5** **Cell 6** **Cell 7** **Cell 8** **Cell 9** **Cell 10**

| | | |
|---|---|---|
| Cell-1 | Proteinase K solution | 50 µl |
| Cell-2 | Lysis Buffer 2 | 1500 µl |
| Cell-3 | Binding Buffer 1 | 1000 µl |
| Cell-4 | Magnetic Bead Solution | 1000 µl |
| Cell-5 | Washing Buffer 1 | 1500 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | Elution Buffer 1 | 1000 µl |
| Cell-9 | Elution Buffer 2 | 1000 µl |
| Cell-10 | Empty | |

### 5.6. Storage

BioMagPure Blood DNA Extraction Kit 1200 should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition

Store the purified total nucleic acid at 4 °C (short-term, less than 10 days) or aliquot and store at –70°C (long-term) before perform the downstream analysis.

### 5.7. Starting material

| | |
|---|---|
| Sample type | Large volume of mammalian whole blood, buffy coat, leukocyte concentration |
| Target nucleic acid | Total DNA (Genomic DNA, mitochondrial and/or viral DNA) * |
| Sample volume | 400-1000 µl whole blood (WBC count less than 4 x $10^4$ cells/ µl); |
| | 400-1000 µl buffy coat; |
| Controls / optional internal control ** | Add controls /internal control in the extraction procedure if the downstream analysis needed |
| Elute volume | 100-400 µl*** |

BioMagPure Blood DNA Extraction Kit has proven to work for fresh or frozen blood samples collected in tubes containing common anti-coagulants like EDTA, heparin**** and citrate.

If the sample volume is less than 400 µl or the WBCs no. is high (more than 40000 cells/ µl), add an appropriate volume of PBS to adjust.

This protocol were established for isolating DNA form whole blood of healthy individual, the unhealthy or drug-treated individual (e.g. Patients of leukaemia or infection disease) may show abnormal blood quality that may influence the nucleic acid extraction procedure

Using fresh whole blood sample (within 1 week, stored at 4-8℃) for extraction is recommended, the total nucleic acid yield and quality would be decreased by time. For longer storage time, whole blood should be frozen and avoid freeze-thaw

For short-term storage (up to 10 days), store the tubes at 2–8°C. However, for applications requiring maximum fragment size, such as Southern blotting, storage purified DNA at 2–8°C for up to 3 days, as low levels of DNA degradation will occur after this time

For long-term storage, store the purified DNA at –70°C.

In fact, the eluate contains total nucleic acid (DNA and RNA), the RNA is not the major product in this kit (about 10%) and would be degraded soon. If the RNA-free product is needed, add some RNase to the eluate.

### 5.8. Expected purity and yield

DNA was purified by using the BioMagPure Blood DNA extraction Kit 1200 and the BioMagPure Purification Instrument using four different blood samples. The DNA concentration was measured using a NanoDrop® 2000 spectrophotometer. The range of DNA yield is 2-200 µg (in the WBCs range 2-40 x $10^3$ cells/ µl). The DNA yield from whole blood depended on the specific blood donor and blood cell count. The yield from cultured cell depended on the type of cell line due to the variable degree of aneuploidy.

| Sample material | Volume / amount | DNA Yield | Purity |
|---|---|---|---|
| Whole blood (WBC count 6 x $10^3$/ml) | 400 µl<br>1000 µl | 9.5-11.5 µg<br>25-30 µg | OD260/OD280 >=1.7<br>OD260/OD230 >=1.5 |
| Whole blood (WBC count 11 x $10^3$/ µl) | 400 µl<br>1000 µl | 18-19 µg<br>45-55 µg | |
| Whole blood (WBC count 25 x $10^3$/ µl) | 400 µl<br>1000 µl | 35-40 µg<br>80-100 µg | |
| Whole blood (WBC count 40 x $10^3$/ µl) | 1000 µl | 120-180 µg*** | |
| Buffy coat | 100 µl<br>200 µl | 10-30 µg<br>20-60 µg | |

### 5.9. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 5.10. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Blood DNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

* If the WBC count of the blood sample is more than 4 x $10^4$ cells/ µl, we recommend diluting the blood sample with PBS or saline
** See Controls / internal control below
*** For samples with WBC count higher than 30000 cells/ µl, we recommend setting elute volume higher than 200 µl
**** The EDTA is recommended to use as anticoagulation agent, while heparin have inhibit effects on nucleic acid amplification reaction

# 6. BioMagPure Viral Nucleic Acid Extraction Kit

Cat. No. BS-060201-CK
Process time: BioMagPure 12S – 40-55 minutes
BioMagPure 24 – 40-60 minutes

### 6.1. Intended use

BioMagPure Viral Nucleic Acid Extraction Kit is used with the BioMagPure instrument for extraction of Viral DNA or RNA from human biological specimens such as serum, plasma, and other cell-free fluids.

### 6.2. Application

Nucleic acids extracted from BioMagPure Viral Nucleic Acid Extraction kit can be used in a number of downstream application including: PCR, qPCR, Sequencing(NGS), Microarray, RFLP, Southern Blot Analysis.

### 6.3. Number of tests

48 extractions

### 6.4. Kit components

| Kit Contents | BS-060201-CK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| RNA Carrier (1mg) | 1 pce. |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 6.5. Reagent cartridge contents

| Cell 1 | Cell 2 | Cell 3 | Cell 4 | Cell 5 | Cell 6 | Cell 7 | Cell 8 | Cell 9 | Cell 10 |
|---|---|---|---|---|---|---|---|---|---|

| | | |
|---|---|---|
| Cell-1 | Proteinase K solution | 30 µl |
| Cell-2 | Lysis Buffer 4 | 720 µl |
| Cell-3 | Binding Buffer 1 | 1000 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 2 | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | RNase-free water | 1000 µl |
| Cell-9 | RNase-free water | 1000 µl |
| Cell-10 | Empty | |

### 6.6. Storage

BioMagPure Viral Nucleic Acid Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition.

After dissolve the RNA carrier, store it at 4°C (short-term, up to 1 month) or -20°C (long-term). Do not freeze–thaw a frozen RNA carrier more than 3 times. Store the nucleic acid at 4°C (up to 24 hours) or -20°C for longer storage. Repeated freeze–thawing is not allowed.

### 6.7. Starting material

| Sample Type | Target Nucleic Acid | Sample Volume (Amount of starting material) | Elution Volume |
|---|---|---|---|
| Serum | Total Viral Nucleic Acids (DNA + RNA) | 100-400 µl | 50-300 µl |
| Plasma | | | |
| CSF | | | |
| Pre-treated Urine | | | |
| Cell-free body fluids | | | |
| Controls/internal control* | Add controls /internal control in the extraction procedure if the downstream analysis needed | | |

The kit is designed for extraction of viral nucleic acids (e.g., those of HIV, HCV, HBV, CMV and EBV) from plasma or serum, or from a pool of such cell-free body fluids.

After extraction, store the nucleic acid at 4°C (up to 24hours) or -20°C for longer storage. Repeated freeze–thawing is not allowed.

### 6.8. Sample preparation

The purification procedure is optimized for use with 100-400 µl serum, plasma, CSF, or pre-treated urine samples. (Blood samples treated with EDTA or citrate as an anticoagulant can be used for plasma preparation)

Samples can be either fresh or frozen, provided that they have not been refrozen after thawing

After collection and centrifugation, plasma, serum, or CSF can be stored at 2–8°C for up to 6 hours. For longer storage, we recommend freezing aliquots at –20°C or –80°C. Thaw samples at room temperature (15–25°C), and process the samples immediately when they have equilibrated to room temperature. Do not refreeze the aliquots after thawing. Repeated freeze–thawing leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids. If cryoprecipitates are visible in the samples, centrifuge at 6800 x g for 3 minutes, transfer the supernatants to fresh tubes without disturbing the pellets, and start the purification procedure immediately.

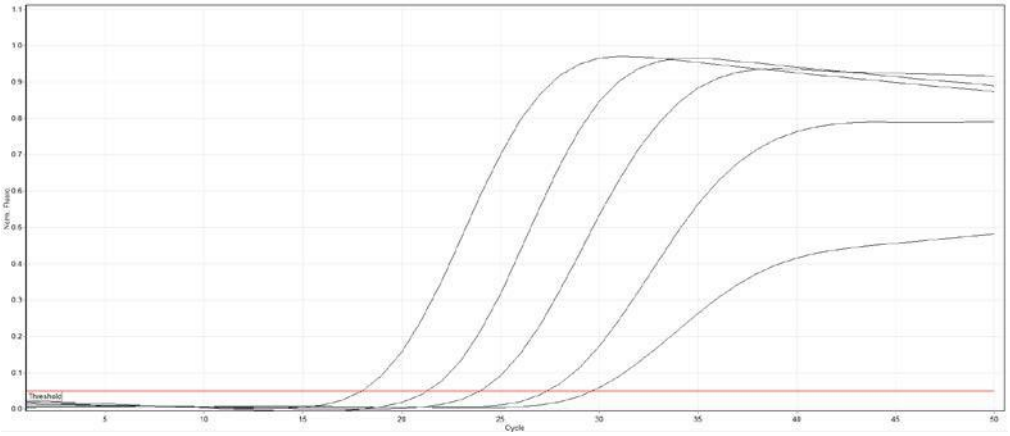
### 6.9. RNA carrier

For RNA virus, adding RNA to the sample before extraction is recommended!!!

Add 1.0 ml RNase- free water to the RNA carrier tube (provided with the kit) and mix by vortexing. Store it at 4°C (short-term, up to 1 month) or -20°C (long-term). Do not freeze–thaw the Frozen RNA carrier more than three times. Add 5 µl RNA carrier (for 100 µl sample), 10 µl (for 200 µl sample) or 20 µl (for 400 µl sample) into the sample tube before add sample

### 6.10. Result

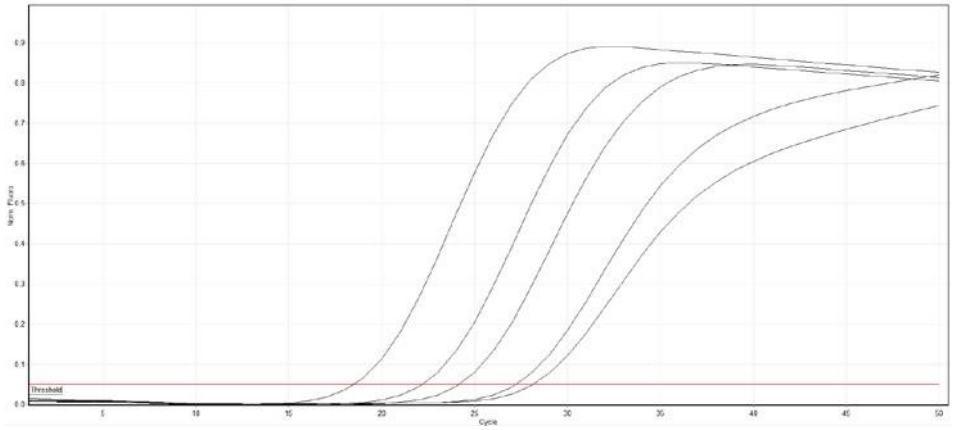
#### 6.10.1. DNA virus (HBV)



Using serum spiked with serial-diluted Hepatitis B Virus (in range of 300000-30 IU/ml). 200 µl sample were extracted and eluted in 100 µl. 30 µl eluate was used for real-time PCR reaction by AmpliSens® HCV/HBV/HIV-FRT PCR kit. As little of less than 6 IU spiked (about 1 IU in PCR reaction) sample can be detected, proving the excellent sensitivity and linearity of isolation procedure.

* See Controls / internal control below

### 6.10.2. RNA virus (HCV)



Using serum spiked with serial-diluted Hepatitis C Virus (in range of 500000-50 IU/ml). 200 µl sample were extracted and eluted in 100 µl. 30 µl eluate was used for real-time PCR reaction by AmpliSens® HCV/HBV/HIV-FRT PCR kit. As little of less than 10 IU spiked (about 3 IU in PCR reaction) sample can be detected, proving the excellent sensitivity and linearity of isolation procedure.

## 6.11. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

## 6.12. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Viral DNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

# 7. BioMagPure Tissue DNA Extraction Kit

Cat. No. BS-060201-DK
Process time: BioMagPure 12S – 45-55 minutes
BioMagPure 24 – 45-60 minutes

### 7.1. Intended use

BioMagPure Tissue DNA Extraction Kit is used with the BioMagPure instrument for extraction of genomic DNA from a variety of animal tissues, swab samples and blood stain

For extraction from FFPE samples, using "BS-060201-JK BioMagPure FFPE DNA extraction kit" is recommended

### 7.2. Application

Nucleic acids extracted from BioMagPure Tissue DNA Extraction kit can be used in a number of downstream application including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, Southern Blot Analysis.

### 7.3. Number of tests

48 extractions

### 7.4. Kit components

| Kit Contents | BS-060201-DK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| Proteinase K (10mg/mL) | 1 pce. (1 ml) |
| BL2 Buffer | 1 pce. (25 ml) |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 7.5. Reagent cartridge contents

| Cell 1 | Cell 2 | Cell 3 | Cell 4 | Cell 5 | Cell 6 | Cell 7 | Cell 8 | Cell 9 | Cell 10 |
|---|---|---|---|---|---|---|---|---|---|

| | | |
|---|---|---|
| Cell-1 | Empty | |
| Cell-2 | Lysis Buffer 3 | 720 µl |
| Cell-3 | Binding Buffer 1 | 720 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 1 | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | Elution Buffer 1 | 1000 µl |
| Cell-9 | Elution Buffer 2 | 1000 µl |
| Cell-10 | Empty | |

### 7.6. Storage

BioMagPure Tissue DNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition

Store the purified total nucleic acid at 4 °C (short-term, less than 10 days) or aliquot and store at –70°C (long-term) before perform the downstream analysis.

### 7.7. Starting material

The types and amounts of starting material for use in BioMagPure Tissue DNA purification procedures are shown in Table listed below.

| Sample type | Target nucleic acid | Sample volume (amount of starting material) | Elution volume |
|---|---|---|---|
| Tissue | DNA | 100-400 µl/10-40 mg | 50-300 µl |
| Dried swab samples (e.g. Buccal cells) | | 100-400 µl/1 swab or brush (add BL2 and proteinase K to100-400 µl for extraction) | |
| Dried blood | | 100-400 µl/4 discs* | |
| Control/Optional internal control** | Add controls /internal control in the extraction procedure if the downstream analysis needed | | |

### 7.8. Yield of purified DNA

DNA yields depend on the sample type, number of nucleated cells in the sample, and the protocol used for purification of DNA.

Table listed below shows DNA yields obtained from different sample types using BioMagPure extraction procedures.

| Sample Type | Sample Amount | Typical DNA Yield |
|---|---|---|
| Skeletal muscle | 200 µl (40 mg tissue digested) | Up to 9 µg |
| Heart | 200 µl (20 mg tissue digested) | Up to 12 µg |
| Spleen | 200 µl (10 mg tissue digested) | Up to 27 µg |
| Lung | 200 µl (10 mg tissue digested) | Up to 17 µg |
| Kidney | 200 µl (10 mg tissue digested) | Up to 18 µg |
| Liver | 200 µl (10 mg tissue digested) | Up to 40 µg |
| Buccal cells | 1 swab | 1-5 µg |
| Dried blood | 4 x 3 mm diameter discs | 0.2-0.5 µg |

Sample preparation requirements are highly dependent upon the type of starting material. Due to variations in consistency and viscosity, even similar sample types may require distinct handling. The steps below describe some recommendations for processing primary samples.

### 7.9. Sample preparation

To extract DNA from FFPE samples, please select and refer to BioMagPure FFPE DNA Extraction Kit (BS-060201-JK). Efficient disruption and homogenization of sample material is essential for isolation of genomic DNA from tissue. On the other hand, too extensive disruption and homogenization will lead to shearing of high-molecular weight genomic DNA. Always freshly prepare tissue lysate and process them immediately. Store the lysate at-15 to -20 ˚C or below when DNA isolation is to be postponed. To deal with the RNA-rich tissue (e.g. High gene expression tissue, such as liver and tumour), add RNase after Proteinase K incubation to digest the RNA and increase the DNA yield

<table>
<tr><td colspan="2">For solid animal tissues</td></tr>
<tr><td>1. Transfer tissue</td><td>Transfer tissue into a 1.5 ml microtube:
<table>
<tr><th>No</th><th>Sample type***</th><th>Recommended sample amount****</th></tr>
<tr><td>1</td><td>Heart</td><td>20 mg</td></tr>
<tr><td>2</td><td>Muscle</td><td>40 mg</td></tr>
<tr><td>3</td><td>Other tissues</td><td>10 mg</td></tr>
</table></td></tr>
<tr><td>2. Add BL2 buffer</td><td>Add 220-440 µl of provided BL2 buffer. Ensure that tissue pieces are fully submerged in BL2 buffer</td></tr>
<tr><td>3. Add proteinase K</td><td>Add 20 µl proteinase K solution and mix by vortexing</td></tr>
<tr><td>4. Incubation</td><td>Incubate at 55℃ in a shaking water bath or thermomixer (mix set at 1000 RPM) until the tissue is completely lysed<br><br>Note<br>Lysis time varies depending on the type of tissue processed. Lysis is usually completed in 1-2 hrs. However, lysis overnight is possible and donor not influence the preparation. Use the heat block for incubation, vortexing-mix several times during incubation is recommended.</td></tr>
<tr><td>5. RNAse treatment (optional)</td><td>Inactivate proteinase K by increasing the temperature to 70°C for 10 minutes. Add RNAse A (not supplied to the lysate. Incubate 10 minutes.</td></tr>
</table>

* A 3mm diameter disc punched out from filter paper stained with dried blood contains white blood cells from approximately 5 µl whole blood; we recommend using 4 punched-out discs as starting material.

** See Controls / internal control below

*** Cut the tissue into pieces or use homogenizer will enhance the lysis efficiency and increase the DNA yield

**** If use more amount than recommended, increase the input of BL2/proteinase K may be needed( only in the tissue can't be lysed completed)

| | |
|---|---|
| 6. Spin down and transfer | Spin down and transfer clear supernatant to sample tube. Use BL2 buffer to adjust the sample volume setting. Proceed to tissue DNA extraction |

| For swab samples | |
|---|---|
| 1. Cut | Carefully cut or break off the end part of the swab or brush into a 1.5 ml microcentrifuge tube by using an appropriate tool (e.g. scissors). |
| 2. Add BL2 buffer | Add 220-440 µl of provided BL2 Buffer. Ensure tissue pieces are fully submerged in BL2 Buffer |
| 3. Add proteinase K | Add 20 µl proteinase K solution and mix by vortexing<br>**Note**<br>If processing buccal cell brush samples, centrifuge the tube briefly (at 10000 x g, for 30 s) to force the brush to the bottom of the tube |
| 4. Incubate | Incubate at 55°C in a shaking water bath or thermomixer (mix set at 1000 RPM) until the tissue is completely lysed<br>**Note**<br>Lysis time varies depending on the type of tissue processed. Lysis is usually completed in 1-2 hrs. However, lysis overnight is possible and donor not influence the preparation. Use the heat block for incubation, vortexing-mix several times during incubation is recommended |
| 5. Centrifugate | Briefly spin the tube to remove drops from the lid |
| 6. Remove | Remove the swab or brush from the tube. Using forceps press the swab or brush again the inside of the tube to obtain maximum sample volume. The sample volume should be approximately as the setting volume (200-400 µl) by using BL2 buffer to adjust the volume |
| 7. Transfer | Transfer to sample tube, proceeding tissue DNA extraction |

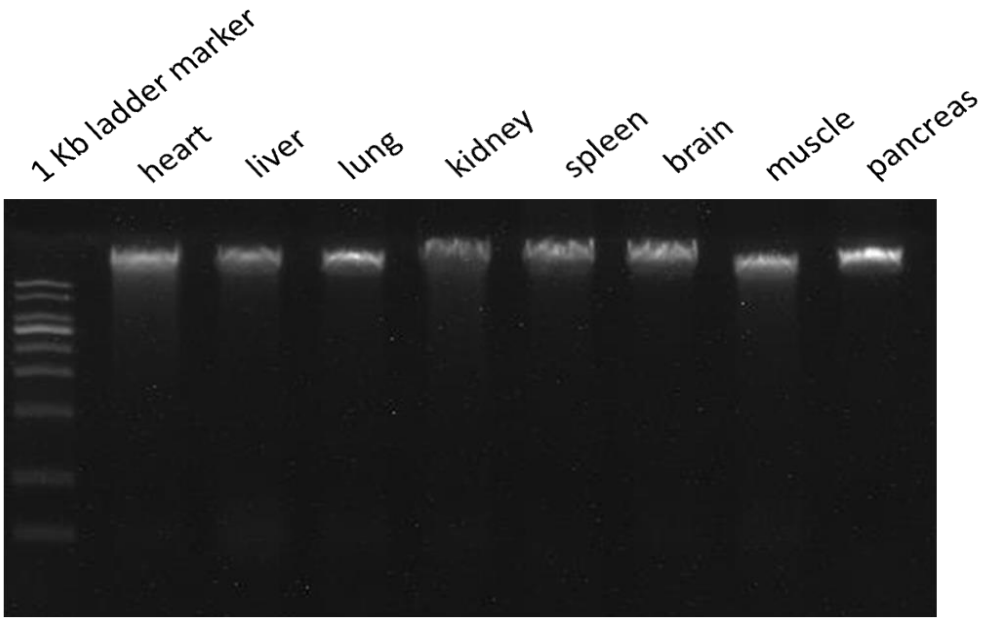
| Dried blood | |
|---|---|
| 1. Collect | Collect 70 µl of each blood sample onto a ring marked on filter paper. Allow the blood to air dry.<br>**Note**<br>Either untreated blood or blood containing anticoagulant (e.g. EDTA,ACD or heparin) can be used |
| 2. Cut | For each dried blood sample, use the manual paper punch to cut out four 3mm diameter discs |
| 3. Add BL2 buffer | Transfer each set of four discs to a 1.5ml microcentrifuge tube. Add 220-440 µl BL2 to sample |
| 4. Add proteinase K | Add 20 µl proteinase K and mix by vortexing |
| 5. Incubate | Incubate at 55℃, 15min in a thermomixer (set at 1000 RPM) or vortex mix several times during incubation in the heat block |
| 6. Centrifugate | Centrifuge the tube briefly to remove the drop inside the lid |
| 7. Transfer | Transfer the 200-400 µl supernatant to the sample tube, proceeding tissue DNA extraction |

### 7.10. Result

#### 7.10.1. Performance

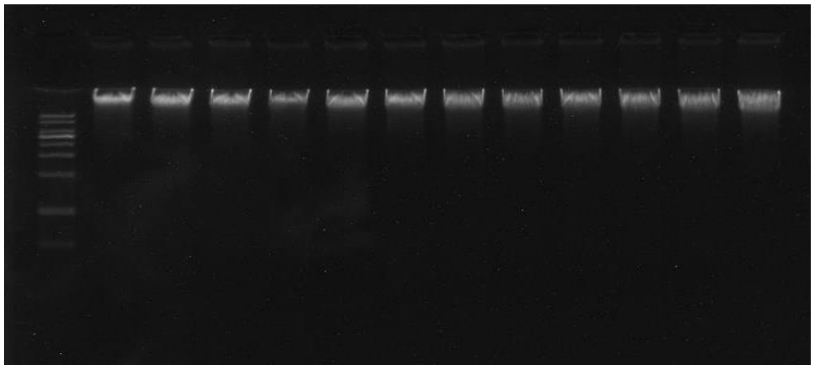
Use BioMagPure Tissue DNA Extraction kit to extract mouse tissues, the results were obtained by Nanodrop 2000 spectrophotometer and TAE agarose gel.

| Tissue type | Total nucleic acid yield (µg) | DNA yield (µg) (After RNase treatment) |
|---|---|---|
| Heart - 10mg | 12-20 | 6-13 |
| Liver - 10mg | 25-40 | 10-20 |
| Lung - 10mg | 7-15 | 9-14 |
| Kidney - 10mg | 30-40 | 20-30 |
| Spleen - 10mg | 20-40 | 15-30 |
| Brain - 10mg | 15-20 | 12-15 |
| Muscle - 10mg | 4-8 | 3-5 |
| Pancreas - 10mg | 3-5 | 3-5 |
| Tail - 10mg | 7-12 | 5-10 |
| Ear - 10mg | 15-20 | 14-20 |



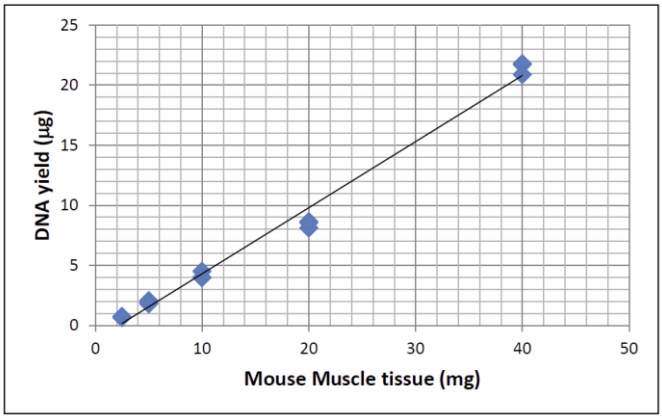

### 7.10.2. Reproducibility

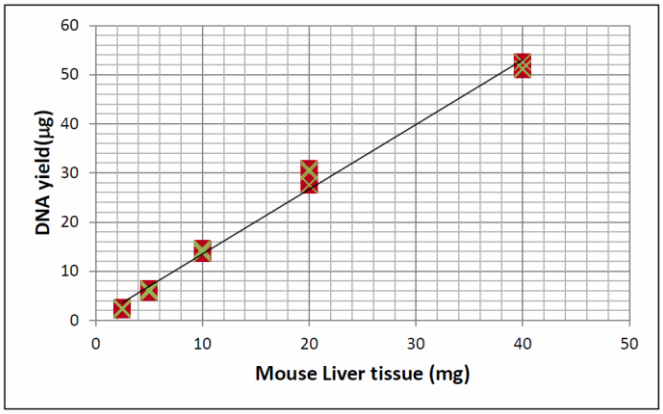
Use twelve 10mg mouse tissue (lung) as samples. Analysis result by TAE agarose gel.



### 7.10.3. Scalability

Use different amount of mouse liver and muscle tissue as samples. Analysis the result by Nanodrop 2000 spectrophotometer.




## 7.11. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

## 7.12. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Tissue DNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

# 8. BioMagPure Cultured Cell DNA Extraction Kit

Cat. No. BS-060201-EK
Process time: BioMagPure 12S – 45-55 minutes
BioMagPure 24 – 45-60 minutes

### 8.1. Intended use

BioMagPure Cultured Cell DNA Extraction Kit is used with the BioMagPure instrument for extraction of genomic DNA from culture cells and buffy coat.

### 8.2. Application

Nucleic acids extracted from Cultured Cell DNA Extraction kit can be used in a number of downstream application including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, Southern Blot Analysis.

### 8.3. Number of tests

48 extractions

### 8.4. Kit components

| Kit Contents | BS-060201-EK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 8.5. Reagent cartridge contents

| Cell 1 | Cell 2 | Cell 3 | Cell 4 | Cell 5 | Cell 6 | Cell 7 | Cell 8 | Cell 9 | Cell 10 |
|---|---|---|---|---|---|---|---|---|---|

| | | |
|---|---|---|
| Cell-1 | Proteinase K solution | 40 µl |
| Cell-2 | Lysis Buffer 3 | 720 µl |
| Cell-3 | Binding Buffer 1 | 720 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 1 | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | Elution Buffer 1 | 1000 µl |
| Cell-9 | Elution Buffer 2 | 1000 µl |
| Cell-10 | Empty | |

### 8.6. Storage

BioMagPure Cultured Cell DNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition.

Store the purified total nucleic acid at 4 °C (short-term, less than 10 days) or aliquot and store at –70°C (long-term) before perform the downstream analysis.

### 8.7. Starting material

#### 8.7.1. Culture cells in suspension and monolayer. Cells from Buffy coat (without red blood cells).

If the buffy coat is remove directly from the whole blood, for completely remove and lyse red blood cell, the BioMagPure Blood DNA Extraction kit (BS-060201-AK; BS-060201-BK) is recommended.

Do not use more than 5 x 106 cells with a normal set of chromosomes.

The cell number can be determined by using Hemocytometer1,2 (Petroff-Hauser Chamber) and automated cell counter (e.g.TC10™, Countess®, Cellometer®, and Scepter™ automated cell counters).

The types and amounts of starting material for use in BioMagPure Cultured Cell DNA purification procedures are shown in Table listed below.

Reference

**http://www.smccd.edu/accounts/case/biol230/algae/hemocytometer1.pdf**
**http://web.mnstate.edu/provost/CountingCellsHemocytometer.pdf**

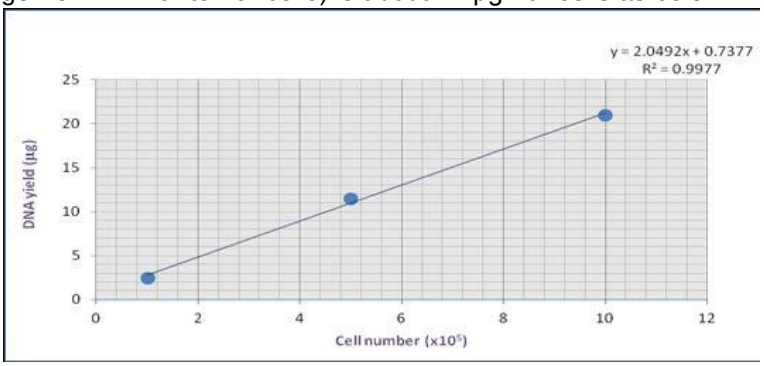
### 8.8. Sample preparation

| | |
|---|---|
| Cells grown in suspension | **Suspension culture**: Determine the number of cells. (Do not use more than 5 x $10^6$ cells with a normal set of chromosomes). Centrifuge the appropriate number of cells for 5 min at 1000 x g in a 1.5 ml microcentrifuge tube. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Resuspend cell pellet in PBS to a final volume of 200 µl |
| Cells grown in a monolayer | Cells grown in a monolayer can be detached from the culture flask by either trypsinization or using a cell scraper |
| | **To trypsinize cells:** Determine the number of cells. Do not use more than 5 x $10^6$ cells with a normal set of chromosomes). Aspirate the medium and wash cells with PBS. Aspirate the PBS, and add 0.10–0.25% trypsin, incubate at 37°C. After cells have detached from the dish or flask, collect them in medium, and transfer the appropriate number of cells to a 1.5 ml microcentrifuge tube. Centrifuge for 5 min at 1000 x g. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Resuspend cell pellet in PBS to a final volume of 200 µl. |
| | **Using a cell scraper:** Determine the number of cells. Do not use more than 5 x $10^6$ cells with a normal set of chromosomes. Detach cells from the dish or flask by scraping. Harvest and transfer cells to a 1.5 ml microcentrifuge tube and centrifuge for 5 min at 1000 x g. Remove the supernatant completely and discard, taking care not to disturb the cell pellet. Resuspend cell pellet in PBS to a final volume of 200 µl. |

### 8.9. Yield of purified DNA

DNA yields depend on the sample type, number of nucleated cells in the sample, and the protocol used for purification of DNA.

For example, the average DNA yield form the HT29 colon adenocarcinoma cell line at the different concentrations (in the range from 1 x $10^5$ to $10^6$ cells) is about 22 µg/$10^6$ cells as below.




### 8.10. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Cultured Cell DNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

# 9. BioMagPure Bacterial DNA Extraction Kit

Cat. No. BS-060201-FK
Process time: BioMagPure 12S – 55-65 minutes
BioMagPure 24 – 55-75 minutes

### 9.1. Intended use

BioMagPure Bacterial DNA Extraction Kit is used with the BioMagPure instrument for extraction of genomic DNA from both Gram-positive and Gram-negative bacteria.

### 9.2. Application

Nucleic acids extracted from Bacterial DNA Extraction kit can be used in a number of downstream application including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, Southern Blot Analysis.

### 9.3. Number of tests

48 extractions

### 9.4. Kit components

| Kit Contents | BS-060201-FK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| BL2B Buffer (25 mL) | 1 pce. |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 9.5. Reagent cartridge contents

| Cell 1 | Cell 2 | Cell 3 | Cell 4 | Cell 5 | Cell 6 | Cell 7 | Cell 8 | Cell 9 | Cell 10 |
|---|---|---|---|---|---|---|---|---|---|

| | | |
|---|---|---|
| Cell-1 | Proteinase K solution | 40 µl |
| Cell-2 | Lysis Buffer 3 | 720 µl |
| Cell-3 | Binding Buffer 1 | 720 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 1B | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | Elution Buffer 1 | 1000 µl |
| Cell-9 | Elution Buffer 2 | 1000 µl |
| Cell-10 | Empty | |

### 9.6. Storage

BioMagPure Bacterial DNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition.

Store the purified total nucleic acid at 4 °C (short-term, less than 10 days) or aliquot and store at –70°C (long-term) before perform the downstream analysis.

### 9.7. Starting material

Bacterial pellet/colony from culture, cell-free body fluids, liquid transport media, urine, environment material (water, soil, etc.). Use the paraffin- embedded tissue sections as samples, we recommend to extract DNA by BioMagPure FFPE DNA Extraction kit (BS-060201-JK). Use tissue as samples, we recommended use the BioMagPure Tissue DNA Extraction kit.

The types and amounts of starting material for use in BioMagPure Bacterial DNA purification procedures are shown in Table listed below:

| Sample Type | Target Nucleic Acid | Sample volume (Amount of starting material) | Elution Volume |
|---|---|---|---|
| Bacteria Pellet | Genomic DNA | 200-400 µl /Up to 109 bacteria (about OD600 = 3) | 50-300 µl |
| Bacterial colony | | 200-400 µl /1-3 colony | |
| Tissue | | 200-400 µl /1-30 mg | |
| Urine | | 200-400 µl /5-50 mL urine | |
| Cell-free body fluids | | 200-400 µl cell-free body fluids | |
| Liquid transport media | | 200-400 µl liquid transport media | |
| NOTE: Before extraction, adjust sample volume with BL2B buffer | | | |

### 9.8. Sample preparation

Sample preparation requirements are highly dependent upon the type of starting material. Due to variations in consistency and viscosity, even similar sample types may require distinct handling.

The buffer BL2B is specialized for bacterial cell wall lyse (Supplied in the kit), use it to resuspend the bacterial pellet before process extraction. For mycobacterium spp. (e.g. MTB), use buffer BL3 for bacterial cell wall lysis (BL3 buffer is supplied in the BS-060201-IK BioMagPure TB DNA Extraction kit).

For some specimen type (e.g. stool, animal tissue) or bacterial (e.g. Gram-positive bacteria), using homogenizer before extraction. It will help the cell wall breakage and increase the DNA yield

The table below describes the recommendations in processing the primary samples before nucleic acid extraction:

**Table: Preparation of sample material for bacterial nucleic acid extraction**

| Sample type | Procedure |
|---|---|
| For viscous samples<br>e.g. BAL, sputum or other mucous specimen | Recommended pre-treatment : Liquefaction<br>Prepare a fresh DTT stock solution for liquefaction (e.g., 5× conc. DTT stock is about 0.75%). The liquefaction could be done by using other solutions, such as NALC(N-Acetyl-L-Cysteine)-NaOH or other agents which could digest mucous material.<br>Adjust the final DTT concentration in the sample to0.15% by adding DTT stock solution.<br>Incubate the sample (e.g., with shaking at 850 RPM for 30 min at 37°C) until it can be pipette easily.<br>Pellet bacteria by centrifugation at14000 xg for 10 min<br>Discard supernatant, resuspend the pellet in 220 µl Buffer BL2B<br>Transfer 200 µl suspension to sample tube (Supplied in the kit) |
| For large volume liquid samples that have low or unknown bacterial loads<br>e.g. urine, water collected from pool/river stream/tower | Recommended pre-treatment : Centrifugation<br>Centrifuge the sample for up to 10 min at 20000 × g to concentrate the bacterial cells in pellet. If there were sand or other visible particle in the pellet, centrifuge again after BL2B buffer treatment or filter out the dust is recommended.<br>Discard supernatant, resuspend the pellet in 220 µl Buffer BL2B*<br>Take 200 µl suspension to sample tube (Supplied in the kit) |
| For cell-free body fluids<br>(e.g. CSF, BAL, aspirates) | Recommended pre-treatment : Centrifugation<br>**Method 1**<br>Pellet bacteria by centrifugation at14000 x g for 10 min<br>Resuspend bacterial pellet in 220 µl Buffer BL2B<br>Take 200 µl suspension to sample tube (Supplied in the kit)<br>**Method 2 - Centrifugation free**<br>Take 200 µl sample in a 1.5 ml centrifuge tube<br>Add 200 µl buffer BL2B to sample (1:1)<br>Vortex-mixing for 5-10sec<br>Transfer 400 µl sample to sample tube (Supplied in the kit) |
| For swab samples<br>e.g. eye, nasal, pharyngeal, or other swabs | **Method 1**<br>Collect samples and place in 2 ml PBS containing a common fungicide.<br>Incubate for 30min at room temperature<br>Pellet bacteria by centrifugation at14000 x g for 10 min<br>Resuspend bacterial pellet in 220 µl Buffer BL2B (Supplied in the kit)<br>Take 200 µl suspension to sample tube (Supplied in the kit)<br>**Method 2 - centrifuge free**<br>Place the sample swab in 440 µl buffer BL2B, incubate for 30 min at room temperature<br>Transfer 400 µl to sample tube |

| | |
|---|---|
| For some gram-positive bacteria species.<br>Especially for samples that contain particles, e.g. stool | Recommended pre-treatment : Mechanical homogenization<br>Follow the regular homogenization procedures in the laboratory.<br>For some sample types, DNA yield can be improved by performing this homogenization step prior to add buffer BL2B and proteinase K |
| Isolation of genomic DNA from bacterial suspension cultures | Pipet 1 ml of bacterial culture into a 1.5 ml microcentrifuge tube and centrifuge at 5000xg for 5 min<br>Discard supernatant<br>Add 220 µl Buffer BL2Bto pellet and mix by vortexing for 5-10 sec<br>Take 200 µl suspension to sample tube (Supplied in the kit) |
| Isolation of genomic DNA from bacterial plate culture | Take 1-3 bacterial colony from culture plate with an inoculation loop and suspend in 220 µl of buffer BL2B by vigorous stirring<br>Take 200 µl suspension to sample tube (Supplied in the kit) |
| To inactive pathogenic organisms in the sample | Recommended pre-treatment : Boiling<br>Incubate samples at 95°C for 10 min<br>Centrifuge briefly to collect the complete sample volume at the bottom of the tube.<br>Allow samples to cool down or chill on ice, then transfer 100-400 µl cooled sample to the sample tube |

### 9.9. Yield of purified DNA

DNA yields depend on the sample type, number of bacteria in the sample, and the protocol used for purification of DNA.

### 9.10. Result

#### 9.10.1. Scalability

Using BioMagPure Bacterial DNA Extraction kit to isolate the DNA from cultured Escherichia. coli (ATCC25922) and Staphylococcus aureus (ATCC27154) in LB broth at different bacterial density (measure the Optical Density at 600nm; OD600). Take 200 µl bacterial culture for extraction and collect the eluate in 100 µl. The total nucleic acid yield of different bacterial density was measured by Nanodrop 2000 UV-Vis spectrophotometer (fig.1a and 2a) and analysed by 1% TAE agarose gel electrophoresis (fig.1b and 2b). The result shown the nucleic acid extraction in both Gram-negative (E. coli) and Gram-positive (S. aureus) were have excellent scalability.

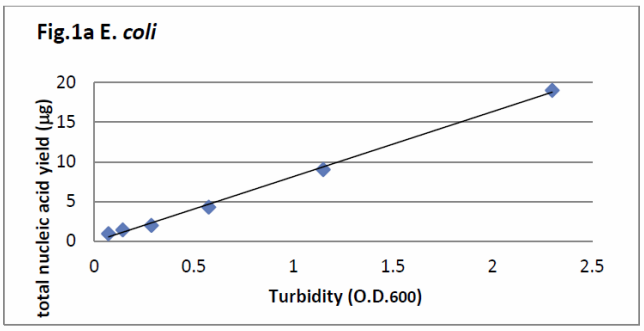
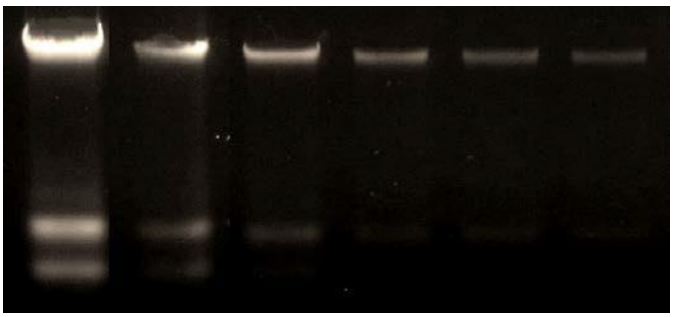



**Fig. 1б**



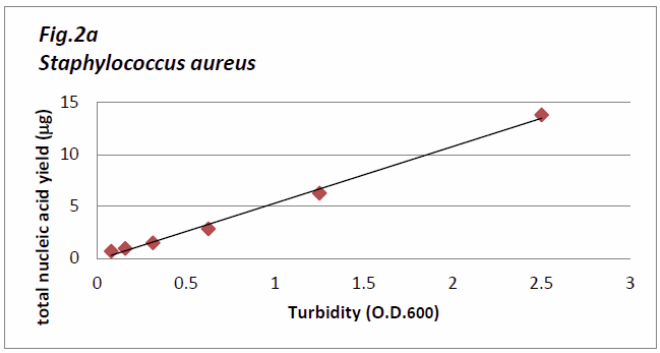
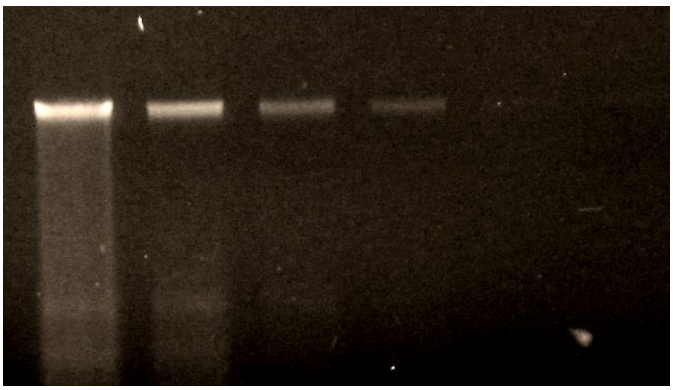



**Fig. 2б**



### 9.10.2. Sensitivity

Performing serial-dilution on Staphylococcus aureus (ATCC27154) in range of 109-101copy/ml). 200 µl sample were extracted and eluted in 100 µl. 25 µl eluate was used for SYBR Green real-time PCR reaction which detect Staphylococcus aureus specific gene. As little of 20 copies (about 102 copy/ml bacteria in the sample) spiked-in (about 5 copy in PCR reaction) bacteria can be detected, proving the excellent sensitivity and linearity of isolation procedure (fig.3a and 3b).

**Fig. 3a**

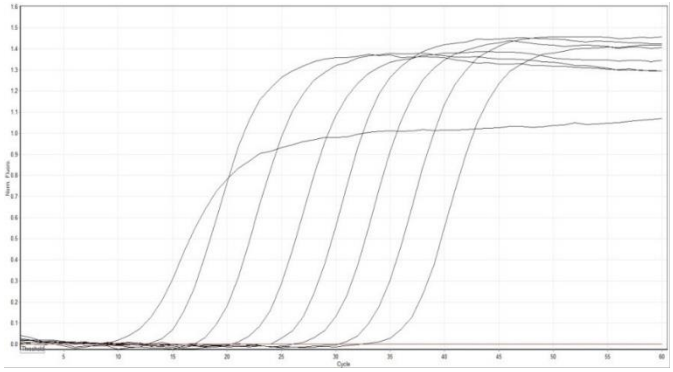
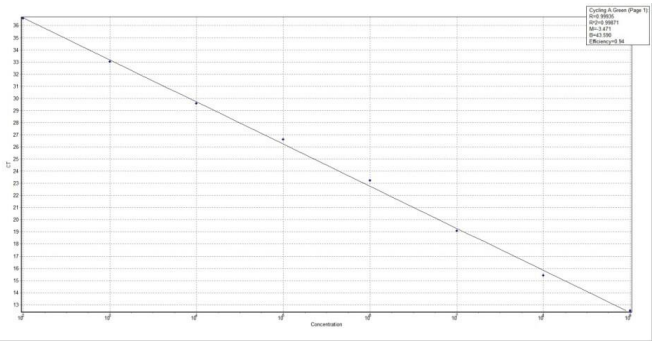


**Fig.3б**



### 9.11. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 9.12. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Bacterial DNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

# 10. BioMagPure HPV DNA Extraction Kit for Swab samples

Cat. No. BS-060201-GK
Process time: BioMagPure 12S – 45-55 minutes
BioMagPure 24 – 45-60 minutes

### 10.1. Intended use

BioMagPure HPV DNA Extraction Kit is used with the BioMagPure instrument for DNA extraction of the Human Papillomavirus (HPV) from cervical cell samples which collected by cervical brush or genital swab in liquid-based Medium* (e.g. Hologic Thinprep PreservCyt®, BD SurepathTM, etc.) or other STM (sample transport media) preservation solutions (e.g. QIAGEN DNA PAP Cervical sampler, Roche Cobas® PCR Cell Collection Media, Hybribio cell preservation solution, etc.).

### 10.2. Application

Nucleic acids extracted from HPV DNA Extraction kit from swab sample can be used in a number of downstream application including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, Southern Blot Analysis, etc.

### 10.3. Number of tests

48 extractions

### 10.4. Kit components

| Kit Contents | BS-060201-GK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| BL4Buffer | 1 pce. (25 ml) |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 10.5. Reagent cartridge contents

**Cell 1** **Cell 2** **Cell 3** **Cell 4** **Cell 5** **Cell 6** **Cell 7** **Cell 8** **Cell 9** **Cell 10**

| | | |
|---|---|---|
| Cell-1 | Proteinase K solution | 40 µl |
| Cell-2 | Lysis Buffer 2A | 720 µl |
| Cell-3 | Binding Buffer 1 | 1000 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 1 | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | Elution Buffer 1 | 1000 µl |
| Cell-9 | Elution Buffer 2 | 1000 µl |
| Cell-10 | Empty | |

* The liquid-base medium are formulated for cellular preservation and used in liquid-based cytological systems (LBC) for cytological and molecular diagnosis

### 10.6. Storage

BioMagPure HPV DNA Extraction Kit for swab samples should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition

Store the purified total nucleic acid at 4 °C (short-term, less than 10 days) or aliquot and store at –70°C (long-term) before perform the downstream analysis.

### 10.7. Starting material

Cervical cells collected by cervical brush or genital swab

For those non-liquid based medium, adding BL4Buffer to the reservation is recommended

The specimen should be sent at 4-30°C for examination on immediately after collection. The storage condition is depended on the preservation solution

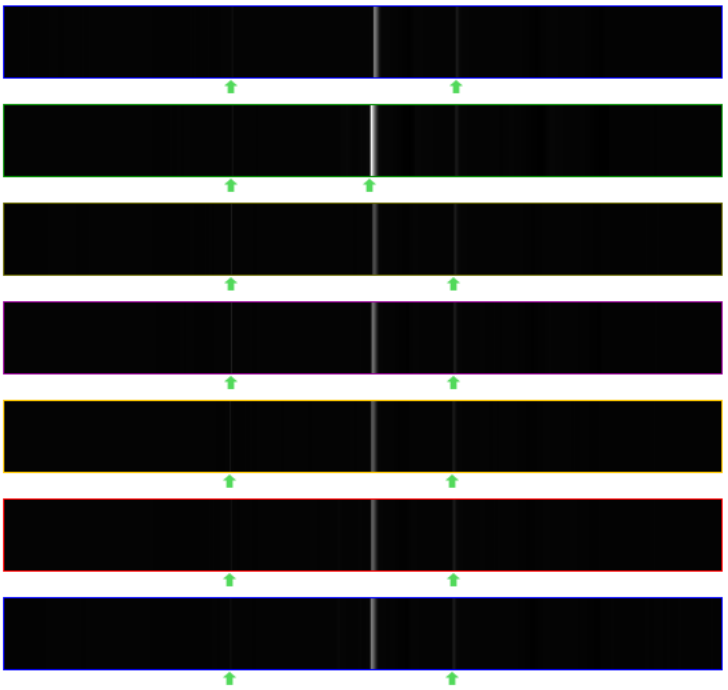
Add controls/internal control in the extraction procedure if the downstream analysis needed (See control/internal control on page 53)

### 10.8. Sample preparation

| Sample type | Procedure |
|---|---|
| In liquid-based preservation solution (e.g. Hologic Thinprep PreservCyt®, BD SurepathTM) | Take the sample amount as the following assay recommended*. Centrifuge at 1000 x g. for 5min. Discard supernatant. Resuspend pellet in 220 µl BL4. Incubate at RT, 5 min. Vortex for 5 s. Take 200 µl suspension for extraction |
| In other STM preservation solution (QIAGEN DNA PAP, Hybribio cell preservation solution) | Add equal volume of BL4 directly to the reservation solution (BL4: reservation = 1:1)** Incubate at RT, 5-10 min. Vortex for 5 s. Take 100-400 µl sample for extraction |

### 10.9. Result

Extraction the DNA from different clinical HPV positive samples. Analysis by the HPV specific PCR and Capillary electrophoresis



### 10.10. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 10.11. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure HPV DNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

* Especially for those assay of "signal amplification", in that the target DNA won't be amplified. Take adequate amount of sample is necessary

** The sticky mucus is common in cervical specimen, adding BL4 before processing will help sample liquefying and nucleic acid extraction

# 11. BioMagPure TB DNA Extraction Kit

Cat. No. BS-060201-IK
Process time: BioMagPure 12S – 60-70 minutes
BioMagPure 24 – 60-75 minutes

### 11.1. Intended use

BioMagPure TB DNA Extraction Kit is used with the BioMagPure instrument for extraction of genomic DNA of Mycobacteria spp. (e.g. *Mycobacterium tuberculosis*) from different specimen.

### 11.2. Application

Nucleic acids extracted from TB DNA Extraction kit can be used in a number of downstream application including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, Southern Blot Analysis.

### 11.3. Number of tests

48 extractions

### 11.4. Kit components

| Kit Contents | BS-060201-IK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| BL3 Buffer | 1 pce. (25 ml) |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 11.5. Reagent cartridge contents

**Cell 1** **Cell 2** **Cell 3** **Cell 4** **Cell 5** **Cell 6** **Cell 7** **Cell 8** **Cell 9** **Cell 10**

| | | |
|---|---|---|
| Cell-1 | Proteinase K solution | 40 µl |
| Cell-2 | Lysis Buffer 3 | 720 µl |
| Cell-3 | Binding Buffer 1 | 720 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 1B | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | Elution Buffer 1 | 1000 µl |
| Cell-9 | Elution Buffer 2 | 1000 µl |
| Cell-10 | Buffer N1 | 400 µl |

### 11.6. Storage

BioMagPure TB DNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition.

Store the purified total nucleic acid at 4 °C (short-term, less than 10 days) or aliquot and store at –70°C (long-term) before perform the downstream analysis.

### 11.7. Starting material

Clinical specimen: Sputum, BAL, Pus, blood, cell-free body fluids, urine and other respiratory specimens

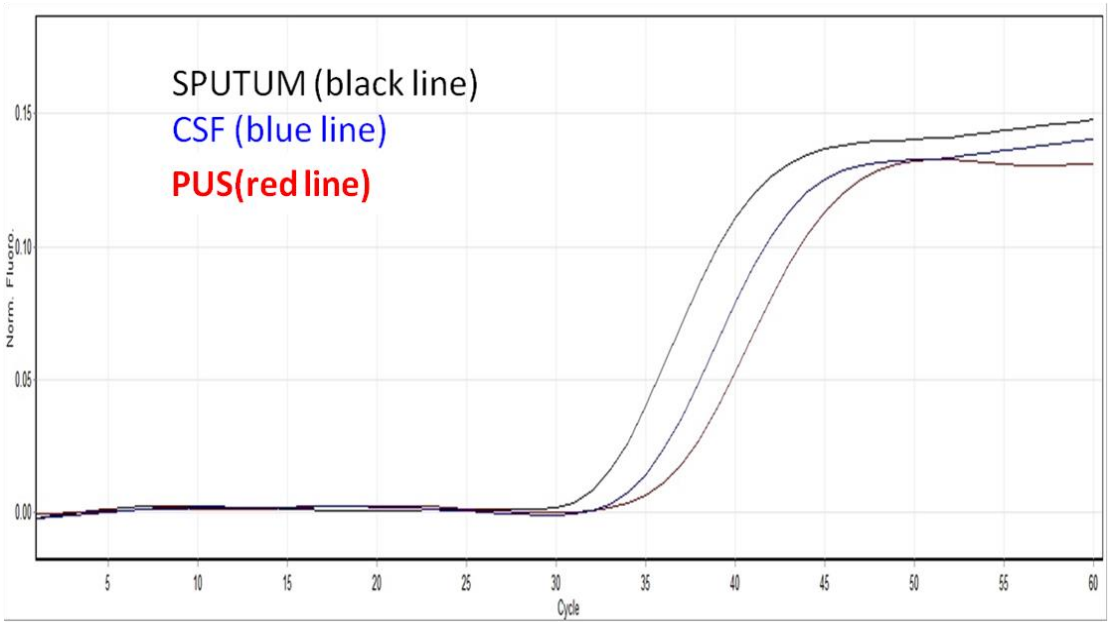
Bacterial culture in the solid and liquid media. For the MTB is highly infectious agent, prepare sample in the BSC (Biosafety cabinet).

### 11.8. Sample preparation

| Specimen type | Procedure |
|---|---|
| Sputum/BAL or other Respiratory Specimen | **Method 1**<br>Liquefy the sample*.<br>Pellet bacteria by centrifugation at 12500 x g for 15 min<br>Discard supernatant, resuspend the pellet in 200 µl Buffer BL3, vortex mixing about 5 sec<br>Take 200 µl sample to sample tube for extraction<br>Method 2 - Centrifuge free<br>Liquefy the sample<br>Transfer 200 µl sample to sample tube<br>Add 200 µl buffer BL3 to sample (1:1) |
| Viscous body fluid<br>e.g. pus | See the procedure of "Sputum/BAL or other Respiratory Specimen" |
| Cell-free body fluid<br>e.g. CSF, urine | Pellet bacteria by centrifugation at14000 x g for 15 min<br>Discard supernatant, resuspend bacterial pellet in 200 µl Buffer BL3,Vortex-mixing about 5 sec<br>Take 200 µl sample to sample tube for extraction |
| Liquefied, decontaminated sample | See the procedure of "cell-free body fluid" |
| Blood or Blood-contaminated sample | Add cold sterilized water to sample to the ratio of water/blood about 3:1<br>Inverted mix several times<br>Incubate at 4°C at least 10 min<br>Centrifuge at 14000 x g for 15 min<br>Remove supernatant, add 200 µl buffer BL3, vortex mixing about 5-10sec<br>Take 200 µl sample to sample tube for extraction |
| Colony from solid culture | Pick 1-3 colony, mix with 200 µl Buffer BL3, vortex mixing about 5-10 sec<br>Take 200 µl sample to sample tube for extraction |
| Liquid culture | **Method 1**<br>Take 1mL culture (＞McFarland 0.5), transfer to 1.5mL microcentrifuge tube<br>Pellet bacteria by centrifugation at 12500 x g for 5 min<br>Discard supernatant, resuspend bacterial pellet in 200 µl Buffer BL3,<br>Vortex mixing about 5-10 sec<br>Take 200 µl sample to sample tube for extraction<br>**Method 2- centrifuge free**<br>Add Buffer BL3 to liquid culture (1:1)<br>Vortex mixing about 5-10 sec<br>Take sample mixture to sample tube for extraction |

### 11.9. Result

Using BioMagPure TB DNA Extraction kit to isolate DNA from clinical specimens (sputum, CSF and pus). 100ml sample used for extraction and collect 100ml eluate. Analysis was performed by real-time qPCR with Taqman probe /primers (IS6110). Even in the cell-free body fluid (CSF) and blood contaminated sample (Pas), the TB DNA can be detected after extraction, proving the excellent sensitivity of isolation procedure.




* The liquefaction could be done by using liquefying agents, such as NALC(N-Acetyl-L-Cysteine)- NaOH and 0.75% DTT (5x stock) which could digest mucous material.

### 11.10. Expected purity and yield

DNA yields depend on the sample type, number of bacteria in the sample, and the protocol used for purification of DNA.

### 11.11. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 11.12. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure TB DNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

# 12. BioMagPure FFPE DNA Extraction Kit

Cat. No. BS-060201-JK
Process time: BioMagPure 12S – 35-45 minutes
BioMagPure 24 – 35-50 minutes

### 12.1. Intended use

BioMagPure FFPE DNA Extraction Kit is used with the BioMagPure instrument for extraction of genomic DNA from FFPE (Formalin-Fixed, Paraffin-Embedded) tissue samples. Providing good quality, high integrity DNA for Molecular diagnosis and research works

### 12.2. Application

Nucleic acids extracted from FFPE DNA Extraction kit can be used in a number of downstream application including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, Southern Blot Analysis

### 12.3. Number of tests

48 extractions

### 12.4. Kit components

| Kit Contents | BS-060201-JK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| Proteinase K (10mg/mL) | 1 pce. (1 ml) |
| BL4 Buffer | 1 pce. (25 ml) |
| DP Buffer | 1 pce. (15 ml) |
| Filter Column | 50 pcs. (50x1) |
| Collection Tube | 50 pcs. (50x1) |
| Barcode Paper | 1 pce. |
| | 1 pce. |

### 12.5. Reagent cartridge contents

**Cell 1** **Cell 2** **Cell 3** **Cell 4** **Cell 5** **Cell 6** **Cell 7** **Cell 8** **Cell 9** **Cell 10**

| | | |
|---|---|---|
| Cell-1 | Empty | |
| Cell-2 | Empty | 720 µl |
| Cell-3 | Binding Buffer 2 | 720 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 5 | 1000 µl |
| Cell-6 | Washing Buffer 2A | 1000 µl |
| Cell-7 | Washing Buffer 2A | 1000 µl |
| Cell-8 | Elution Buffer 1 | 1000 µl |
| Cell-9 | Elution Buffer 2 | 1000 µl |
| Cell-10 | Empty | |

### 12.6. Storage

BioMagPure FFPE DNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition.

Store the purified total nucleic acid at 4 °C (short-term, less than 10 days) or aliquot and store at –70°C (long-term) before perform the downstream analysis.

### 12.7. Starting material

FFPE (formalin fixed Paraffin Embedded) tissue samples: one to five 10 µm-thick section (s)

| Sample Type | Target Nucleic Acid | Sample Volume (Amount of starting material) | Elution Volume |
|---|---|---|---|
| FFPE (formalin fixed Paraffin Embedded) tissue samples | DNA | 200-300 µl/ One to eight 10 µm-thick sections (after proteinase K digestion) * | 50-300 µl |
| Needle biopsy | | 100-400 µl/three to ten biopsies | |

### 12.8. Yield of purified DNA

DNA yields depend on the sample type, number of nucleated cells in the sample, and the thickness of the section

Sample preparation requirements are highly dependent upon the type of starting material. Due to variations in consistency and viscosity, even similar sample types may require distinct handling. The steps below describe some recommendations for processing primary samples

The DNA of FFPE tissue sample is often fragmented which cause problems in molecular assay. Keep the integrity of DNA is most important in whole procedure.

Because of the DNA fragments often cause high OD260 and ratio of 260/280. The integrity of FFPE DNA can't be determined only by UV-VIS spectrophotometer analysis. **The best method for the integrity check is performing a PCR reaction of house-keeping genes with different length products**

### 12.9. Sample preparation

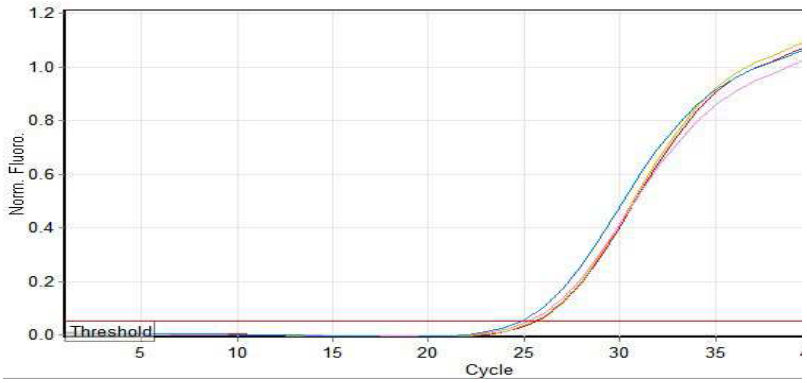
| Transfer sections | Transfer sections 5-10 µm of paraffin-embedded sample into a 1.5/2 ml micro-centrifuge tube |
|---|---|
| Deparaffin | Add 200 µl DP Buffer into micro-centrifuge tube and vortex vigorously for 10 seconds |
| Incubate | Incubate at 56°C for 3 minutes and then allow to cool down to room temperature |
| Add BL4 Buffer | Add 220 µl BL4 buffer and mix by vortexing |
| Centrifuge | Centrifuge at 11000g for 1 minute |
| Add proteinase K | Add 20 µl proteinase K to the lower, blue colour phase directly, and mix gently |
| Incubate | Incubate at 56°C for 2 hours (or until the sample has been completely lysed) |
| Incubate | Incubate at 90°C for 1 hour |
| Transfer | Transfer 200 µl of lower, blue colour phase into filter column sitting in a collection tube. Centrifuge at 6000 xg, 1min. |
| Transfer | Transfer 200 µl of sample into sample tube and start the extraction with BioMagPure system |

### 12.10. Result

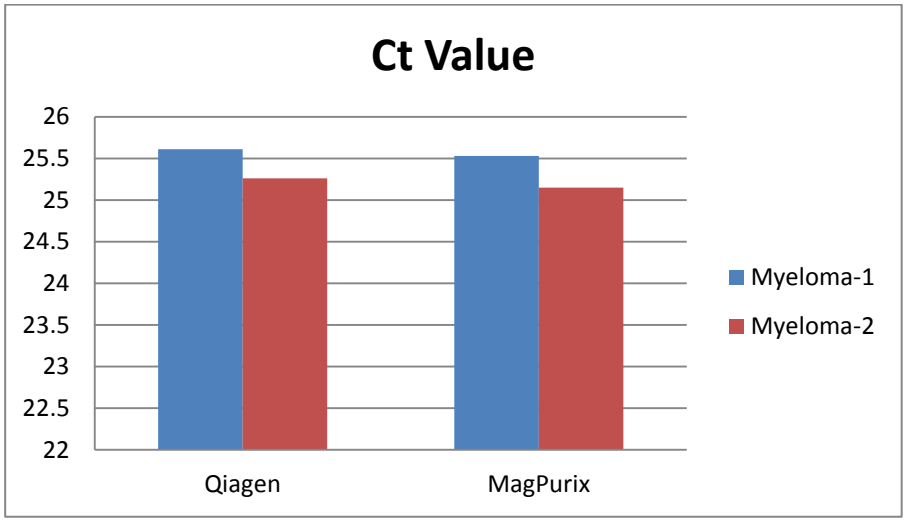
#### 12.10.1. Performance

Take Myeloma FFPE section samples (10 µm thick, about 2 x 2 cm2) for extraction. Two sections were extracted by QIAGEN FFPE Tissue kit; the others were extracted by BioMagPure FFPE DNA Extraction kit. PCR analysis of DNA purified from FFPE tissue samples by using DNA ladder PCR (a multiplex primer PCR of different length products of house-keeping gene, we show one part of the data).

**Fig. 1 the results of Real time PCR shown a good performance of the extract**

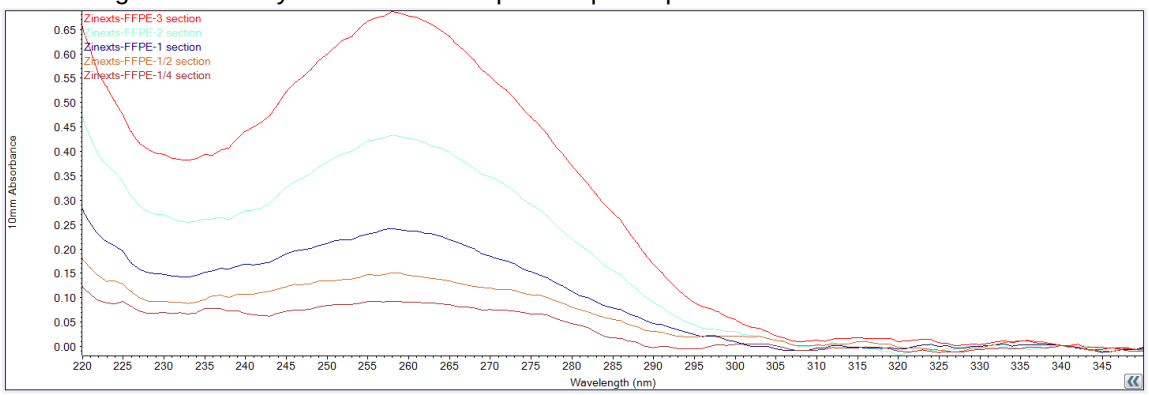


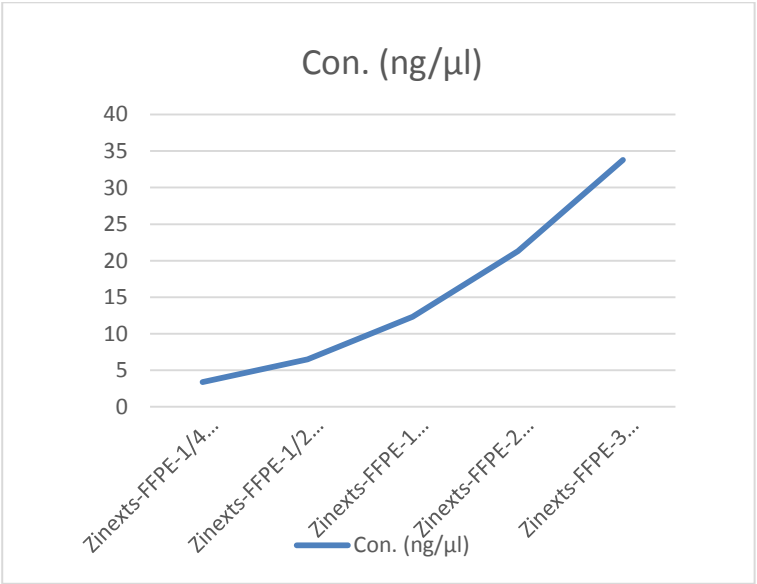

* The size of tissue area should be more than 1 x 1 cm2, if the area is smaller, use more than one section for extraction.




### 12.10.2. Scalability

Using Myeloma FFPE section samples (10 µm thick, about 2 x 2 cm2) for extraction.
Measuring the eluate by UV-VIS Nanodrop2000 spectrophotometer:




### 12.11. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 12.12. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure FFPE DNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

# 13. BioMagPure Forensic DNA Extraction Kit

Cat. No. BS-060201-KK
Process time: BioMagPure 12S – 40-50 minutes
BioMagPure 24 – 40-60 minutes

### 13.1. Intended use

BioMagPure Forensic DNA extraction kit is extract and isolate genomic DNA from forensic samples

### 13.2. Application

The extracted DNA is compatible for use in quantitation using the Quantifiler® Human, Quantifiler® Y Human Male, Quantifiler® Duo DNA Quantification Kits and Investigator® Quantiplex kit, and for use in STR amplification using the AmpFlSTR® PCR Amplification kits

### 13.3. Number of tests

48 extractions

### 13.4. Kit components

| Kit Contents | BS-060201-KK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| Proteinase K (10mg/mL) | 1 pce. (1 ml) |
| BL2 Buffer | 1 pce. (25 ml) |
| Filter Column | 50 pcs. (50x1) |
| Collection Tube | 50 pcs. (50x1) |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 13.5. Reagent cartridge contents

**Cell 1** **Cell 2** **Cell 3** **Cell 4** **Cell 5** **Cell 6** **Cell 7** **Cell 8** **Cell 9** **Cell 10**

| | | |
|---|---|---|
| Cell-1 | Empty | |
| Cell-2 | Lysis Buffer3 | 1000 µl |
| Cell-3 | Binding Buffer 1 | 1000 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 1B | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | Elution Buffer 1 | 1000 µl |
| Cell-9 | Elution Buffer 2 | 1000 µl |
| Cell-10 | Empty | |

### 13.6. Storage

BioMagPure Forensic DNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition

Store the purified total nucleic acid at 4 °C (short-term, less than 10 days) or aliquot and store at –70°C (long-term) before perform the downstream analysis.

### 13.7. Starting material

Whole blood, clotted/dried blood, Forensic Surface and Contact Swabs, hair roots, saliva, sperm stain, chewing gum, cigarette butts, stamps, envelopes, tissue etc.

### 13.8. Sample preparation

| Sample type | Procedure |
|---|---|
| Whole blood (fresh or frozen) | To extract DNA from whole blood samples, please select and refer to BioMagPure Blood DNA Extraction Kit 200 (BS-060201-AK) |
| Clotted/ dried blood | Take 20 µl blood sample to filter paper or bandage. Air-drying the blood sample. Cut the blood-contain range out, transfer pieces to sample tube. Add 400 µl BL2 and 20 µl proteinase K to sample. Incubate at 56°C for 15min, vortex-mixing several times during incubation or place sample in a thermomixer. Transfer all the sample to filter column sitting in a sample tube. Short spin at 500 xg, 1min. Take the sample tube for extraction |
| Forensic Surface and Contact Swabs | Allow the swab or brush to air-dry for at least 2 hours after collection. Carefully cut or break off the end part of the swab or brush into a 1.5ml microcentrifuge tube, using an appropriate tool (e.g., scissors). Add 200 or 400 µl of Buffer BL2 to the sample. Add 20 µl proteinase K, vortex-mixing for at least 10s. If processing brush samples, centrifuge the tube briefly (at 10,000 x g for 30 s) to force the brush to the bottom of the tube. Incubate at 56°C for 15 min. vortex-mixing several times during incubation or place sample in a thermomixer. Transfer all the sample to filter column sitting in a sample tube. Short spin at 500 xg, 1min. Take the sample tube for extraction |
| Hair root | Using two or three 0.5–1 cm from the root ends of plucked hair samples<br>Method 1<br>Place the hair sample in a 1.5 ml microcentrifuge tube. Add 200 µl Buffer BL2 to the sample. Add 20 µl proteinase K and 10 µl 1M DTT solution*, and mix thoroughly by vortexing for at least 10 s. Prepare 1M DTT solution before processing the protocol (1M is about 15% DTT (m/v)). Incubate at 56°C for at least 6 h, vortex-mixing several times during incubation or place sample in a thermomixer. (Optional) Add extra 10 µl proteinase K and 10 µl DTT and incubate at 56°C until the hair samples are completely dissolved. Spin the tube to remove drops from inside the lid. Transfer all the sample to filter column sitting in a sample tube. Short spin at 500 xg, 1min. Take the sample tube for extraction<br><br>Method 2<br>Place the hair sample in a 1.5 ml microcentrifuge tube. Add 200 µl Buffer BL2 to the sample. Add 20 µl proteinase K, mix thoroughly by vortexing for at least 10 s. Incubate at 56°C overnight, vortex-mixing several times during incubation or place sample in a thermomixer. Spin the tube to remove drops from inside the lid. Transfer all the sample to filter column sitting in a sample tube. Short spin at 500 xg, 1min. Take the sample tube for extraction |
| Human tissues | Using up to 40mg tissue. Place tissue sample into a 1.5 ml microcentrifuge tube. Add 200 or 400 µl buffer BL2 and 20 µl proteinase K to the sample, mix thoroughly by vortexing for 10s. Incubate at 56°C for at least 2 hours, vortex-mixing several times during incubation or place sample in a thermomixer. Incubation for longer time (e.g. overnight) isn't making interferences of nucleic acid extraction. Spin the tube to remove drops from inside the lid. Transfer all the sample to filter column sitting in a sample tube. Short spin at 500g, 1min. Take the sample tube for extraction |
| Saliva | Place up to 50 µl saliva in a 1.5ml microcentrifuge tube. Add 200 µl Buffer BL2 to the sample. Add 20 µl proteinase K, and mix thoroughly by vortexing for 10 s. Incubate at 56°C for 15min, vortex-mixing several times during incubation or place sample in a thermomixer. Spin the tube to remove drops from inside the lid. Take 200 µl to sample tube for extraction |
| Sperm stains | Place 5-10 µl or 1cm2of the forensic sample in a 1.5 ml centrifuge tube. Add 200 or 400 µl Buffer BL2 to the sample. Add 20 µl proteinase K, and mix thoroughly by vortexing for 10 s Incubate at 56°C for 15 min, vortex-mixing several times during incubation or place sample in a thermomixer. Spin the tube briefly to remove drops from inside the lid. Transfer all the sample to filter column sitting in a sample tube. Short spin at 500g, 1min. Take the sample tube for extraction |
| Chewing gum | Use of up to 40 mg of chewing gum cut into small pieces is recommended. Place the chewing-gum sample in a 1.5ml microcentrifuge tube. Add 200 µl Buffer BL2 to the sample. Add 20 µl proteinase K, and mix thoroughly by vortexing for 10 s. Incubate at 56°C for 15 min, vortex-mixing several times during incubation or place sample in a thermomixer. Spin the tube briefly to remove drops from inside the lid. Take 200 µl sample to sample tube for extraction |
| Cigarette butts | Use of approximately 1 cm2 paper from the end of the cigarette or filter is recommended. Place the cigarette-butt sample in a 1.5 ml microcentrifuge tube. Add 200 or 400 µl Buffer BL2 to the sample. (Check if the sample has absorbed buffer BL2,if necessary add more Buffer BL2 to the sample). Add 20 µl proteinase K, and mix thoroughly by vortexing for 10 s |

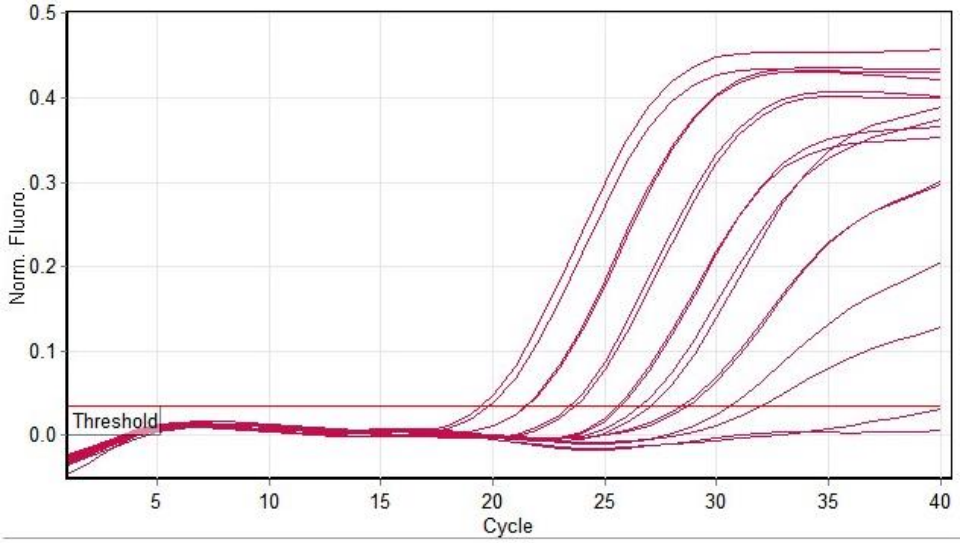
| | |
|---|---|
| | Incubate at 56°C for 15 min, vortex-mixing several times during incubation or place sample in a thermomixer. Spin the tube briefly to remove drops from inside the lid. Take 200 µl sample to sample tube for extraction |
| Stamps, envelopes | Use of a 0.5–2.5 cm2 piece of postage stamp or envelope is recommended. Place all the pieces of sample in a 1.5 ml microcentrifuge tube. Add 200 or 400 µl Buffer BL2 to the sample. (Check if the sample has absorbed buffer BL2, if necessary add more Buffer BL2 to the sample). Add 20 µl proteinase K, and mix thoroughly by vortexing for 10s. Incubate at 56°C for 15 min, vortex-mixing several times during incubation or place sample in a thermomixer. Spin the tube briefly to remove drops from inside the lid. Take 200 µl sample to sample tube for extraction |

### 13.9. Result

#### 13.9.1. Performance

Using QIAGEN Investigator ®Quantiplex to quantification of human genomic DNA. The standard curve (Fig.1A and1B) were made by the standards for genomic DNA concentration calculating. The results were shown as Ct and calculated by the real-time PCR (Table 1).

**Fig. 1A**



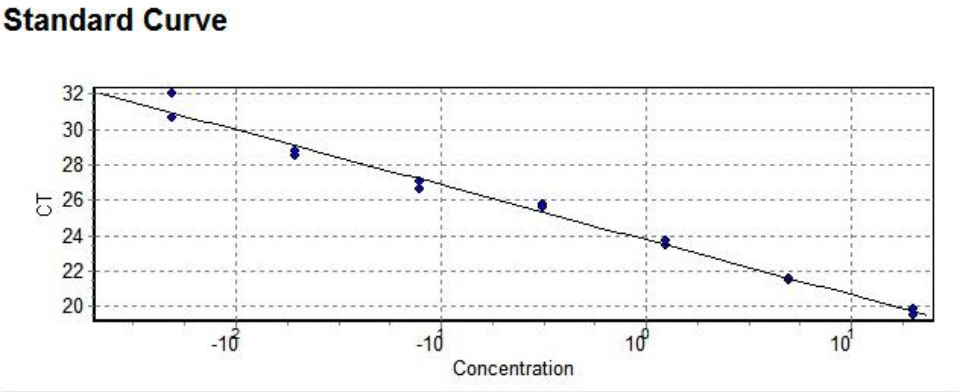




**Fig. 1B**

| Name | Ct | Given Conc (ug/u | Calc Conc (ug/ul) | Rep. Ct |
|---|---|---|---|---|
| Control DNA Z1 20ng/µl | 19.52 | 20 | 23.4924293079773 | 19.70 |
| Control DNA Z1 20ng/µl | 19.89 | 20 | 17.743816822679 | |
| Control DNA Z1 5ng/µl | 21.53 | 5 | 5.28297255153993 | 21.54 |
| Control DNA Z1 5ng/µl | 21.56 | 5 | 5.15506510351862 | |
| Control DNA Z1 1,25ng/µl | 23.72 | 1.25 | 1.03901378233338 | 23.60 |
| Control DNA Z1 1,25ng/µl | 23.48 | 1.25 | 1.23756811894785 | |
| Control DNA Z1 0,3125ng/µl | 25.76 | 0.3125 | 0.228298414974509 | 25.69 |
| Control DNA Z1 0,3125ng/µl | 25.63 | 0.3125 | 0.251925125265275 | |
| Control DNA Z1 0,078125ng/µl | 26.64 | 0.078125 | 0.118599321404503 | 26.86 |
| Control DNA Z1 0,078125ng/µl | 27.08 | 0.078125 | 8.58029166440412E-02 | |
| Control DNA Z1 0,01953125ng/µl | 28.51 | 0.01953125 | 2.97115046127299E-02 | 28.62 |
| Control DNA Z1 0,01953125ng/µl | 28.74 | 0.01953125 | 2.50882331404593E-02 | |
| Control DNA Z1 0,0048828125ng/µl | 32.02 | 0.004882813 | 2.18504909354026E-03 | 31.33 |
| Control DNA Z1 0,0048828125ng/µl | 30.64 | 0.004882813 | 6.08706490645644E-03 | |
| NTC | | | | |
| NTC | | | | |

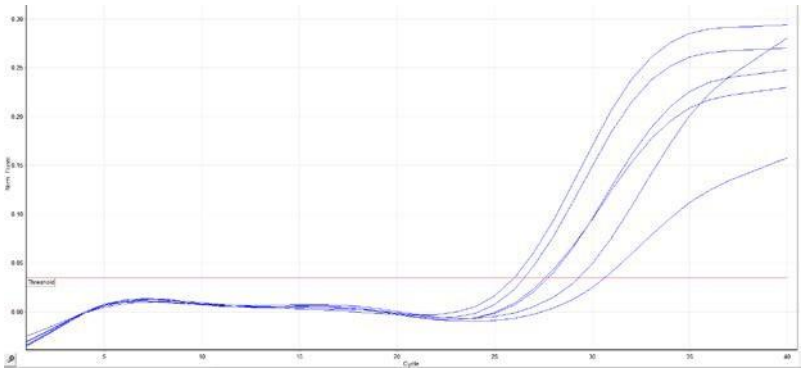
**Table 1**

| Sample type | Results analysis by the investigator® Quantiplex | |
|---|---|---|
| | Ct | Con. (ng/ µl) |
| Oral swab | 21-25 | 3-17 |
| Cigarette butts | 25-26 | 1.2-1.6 |
| Drink surface swab | 27-28 | 0.4-0.5 |
| Straw | 30.5-31 | 0.048 |
| Hair root | 27-38 | 0.0005-0.68 |
| Nail | 27-29 | 0.2-0.7 |
| Dried betel nuts-10mg | 26-27 | 0.9-1.2 |
| Dried blood spot-2 µl* | 29.3-30.6 | 0.01-0.006 |
| Dried blood spot-5 µl* | 27.5-27.8 | 0.051-0.057 |
| Dried blood spot-10 µl* | 26-26.5 | 0.12-0.18 |

### 13.9.2. Scalability

Make different sized blood spot by adding 2, 5, and 10ml blood on filter paper. Air-dried the blood and extracted DNA by BioMagPure Forensic DNA kit. The result were analysed by Investigator® Quantiplex kit:

| Name | Ct | Calc. conc. (µg/µl) | Rep. Ct |
|---|---|---|---|
| NTC | | | |
| NTC | | | |
| Blood spot 2 µl – 5000 bwc | 29.32 | 1.6148677713519E-02 | 29.99 |
| Blood spot 2 µl – 5000 bwc | 30.66 | 5.90729200049097E-03 | |
| Blood spot 5 µl – 5000 bwc | 27.62 | 5.73272999113135E-02 | 27.70 |
| Blood spot 5 µl – 5000 bwc | 27.77 | 5.14260571412719E-02 | |
| Blood spot 10 µl – 5000 bwc | 26.01 | 0.191026109793408 | 26.28 |
| Blood spot 10 µl – 5000 bwc | 26.55 | 0.128416666068004 | |



### 13.10. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 13.11. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Forensic DNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

* Whole blood sample is used, with wbc 500/ µl

# 14. BioMagPure Viral/Pathogen Nucleic Acids Extraction Kit A

Cat. No. BS-060201-LK
Process time: BioMagPure 12S – 40-50 minutes (virus)
BioMagPure 12S – 45-55 minutes (virus + bacteria)
BioMagPure 24 – 40-55 minutes (virus)
BioMagPure 24 – 45-60 minutes (virus + bacteria)

### 14.1. Intended use

BioMagPure Viral/Pathogen Nucleic Acids Extraction Kit A is used with the BioMagPure instrument for extraction of Viral and bacterial DNA/RNA from cell-free samples, such as serum, plasma, and other cell-free body fluids.

### 14.2. Application

Nucleic acids extracted from BioMagPure Viral/Pathogen Nucleic Acids Extraction kit A can be used in a number of downstream application including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, Southern Blot Analysis.

### 14.3. Number of tests

48 extractions

### 14.4. Kit components

| Kit Contents | BS-060201-LK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| RNA Carrier (1mg) | 1 pce. |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 14.5. Reagent cartridge contents

**Cell 1** **Cell 2** **Cell 3** **Cell 4** **Cell 5** **Cell 6** **Cell 7** **Cell 8** **Cell 9** **Cell 10**

| | | |
|---|---|---|
| Cell-1 | Proteinase K solution | 40 µl |
| Cell-2 | Lysis Buffer4 | 720 µl |
| Cell-3 | Binding Buffer 1 | 1000 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 2 | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | RNase-free water | 1000 µl |
| Cell-9 | RNase-free water | 1000 µl |
| Cell-10 | BL2B Buffer | 400 µl |

### 14.6. Storage

BioMagPure Viral/Pathogen Nucleic Acids Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition.

After dissolve the RNA carrier, store it at 4°C (short-term, up to 1 month) or -20°C (long-term). Do not freeze–thaw the Frozen RNA carrier more than3 times.

Store the nucleic acid at 4°C (up to 24hours) or -20°C for longer storage. Repeated freeze–thawing is not allowed.

### 14.7. Starting material

| Sample Type | Target Nucleic Acid | Sample Volume (Amount of starting material) | Elution Volume |
|---|---|---|---|
| Serum | Total bacterial/ Viral Nucleic Acids (DNA + RNA) | 100-400 µl (virus) 100-200 (virus/bacteria) | 50-300 µl |
| Plasma | | | |
| CSF | | | |
| Pre-treated Urine | | | |
| Cell-free body fluids | | | |
| Controls/internal control* | Add controls /internal control in the extraction procedure if the downstream analysis needed | | |

This kit is designed for extraction of viral and bacterial nucleic acids from plasma or serum, or from a pool of such cell-free body fluids.

After extraction, store the nucleic acid at 4°C (up to 24hours) or -20°C for longer storage. Repeated freeze–thawing is not allowed.

### 14.8. Sample preparation

The purification procedure is optimized for use with 100-400 µl serum, plasma**, CSF, pre-treated urine or other cell-free body fluid samples.

Samples can be either fresh or frozen, provided that they have not been refrozen after thawing

RNA Carrier (CARRIER) serves two purposes during the purification procedure. First, it enhances binding of viral nucleic acids to the silica surface of the magnetic particles, especially if the sample contains very few target molecules. Second, the addition of large amounts of RNA Carrier reduces the chances of RNA degradation in the rare event that RNases are not denatured by the chaotropic salts and detergent in the lysis buffer. If RNA carrier is not added to the reaction, recovery of DNA or RNA maybe reduced

After collection and centrifugation, plasma, serum, or CSF can be stored at 2–8°C for up to 6 hours. For longer storage, we recommend freezing aliquots at –20°C or –80°C. Thaw samples at room temperature (15–25°C), and process the samples immediately when they have equilibrated to room temperature. Do not refreeze the aliquots after thawing. Repeated freeze–thawing leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of nucleic acids. If cryoprecipitates are visible in the samples, centrifuge at 6800 x g for 3 minutes, transfer the supernatants to fresh tubes without disturbing the pellets, and start the purification procedure immediately.

### 14.9. RNA Carrier

Add 1.0 ml RNase-free water to lyophilized the RNA Carrier (provided with the kit) and mix by vortexing

Store RNA Carrier at 4°C (short-term, up to 1 month) or -20°C (long-term). Do not freeze–thaw the frozen RNA carrier more than3 times. Divide it into conveniently sized aliquots is recommended

Before nucleic acid extraction, add RNA Carrier to the sample is recommended. Add 5 µl RNA carrier (for 100 µl sample), 10 µl (for 200 µl sample) or 20 µl (for 400 µl sample) into the Sample Tube.

### 14.10. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 14.11. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Viral/Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

* See Controls / internal control below

** Blood samples treated with EDTA or citrate as an anticoagulant can be used for plasma preparation

# 15. BioMagPure Viral/Pathogen Nucleic Acids Extraction Kit B

Cat. No. BS-060201-MK
Process time: BioMagPure 12S – 45-60 minutes
BioMagPure 24 – 45-65 minutes

### 15.1. Intended use

BioMagPure Viral/Pathogen Nucleic Acids Extraction Kit B is used with the BioMagPure instrument for extraction of viral and bacterial DNA/RNA from swab samples (cell-rich samples).

### 15.2. Application

Nucleic acids extracted from BioMagPure Viral/Pathogen Nucleic Acids Extraction kit B can be used in a number of downstream application including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, Southern Blot Analysis.

### 15.3. Number of tests

48 extractions

### 15.4. Kit components

| Kit Contents | BS-060201-MK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| RNA Carrier (1mg) | 1 pce. |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 15.5. Reagent cartridge contents

**Cell 1** **Cell 2** **Cell 3** **Cell 4** **Cell 5** **Cell 6** **Cell 7** **Cell 8** **Cell 9** **Cell 10**

| | | |
|---|---|---|
| Cell-1 | Proteinase K solution | 40 µl |
| Cell-2 | Lysis Buffer 3 | 720 µl |
| Cell-3 | Binding Buffer 1 | 720 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 2 | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | Elution Buffer 1 | 1000 µl |
| Cell-9 | Elution Buffer 2 | 1000 µl |
| Cell-10 | BL2 Buffer | 400 µl |

### 15.6. Storage

BioMagPure Viral/Pathogen Nucleic acids Extraction Kit B should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition

After dissolve the RNA carrier, store it at 4°C (short-term, up to 1 month) or -20°C (long-term). Do not freeze–thaw the Frozen RNA carrier more than3 times.

Store the nucleic acid at 4°C (up to 24hours) or -20°C for longer storage. Repeated freeze–thawing is not allowed.

### 15.7. Starting material

Bacterial pellet/colony from culture, clinical swab samples in liquid transport media, environment material (water, soil, etc.) and other cell-rich samples

Use the tissue or paraffin- embedded tissue sections (FFPE) as samples, we recommend to extract DNA by BioMagPure Tissue DNA Extraction kit (BS-060201-DK)

The types and amounts of starting material for use in BioMagPure Viral/Pathogen Nucleic Acids Extraction kit B purification procedures are shown in Table listed below,

| Sample Type | Target Nucleic Acid | Sample volume (Amount of starting material) | Elution Volume |
|---|---|---|---|
| Bacteria Pellet | Total Viral/Bacterial Nucleic acids (DNA/RNA) | 100-200 µl /Up to 109 bacteria (about OD600 = 3) | 50-300 µl |
| Bacterial colony | | 100-200 µl /1-3 colony | |
| Swab samples | | 100-200 µl liquid transport media | |
| Controls/internal control* | Add controls /internal control in the extraction procedure if the downstream analysis needed | | |

### 15.8. Sample preparation

Sample preparation requirements are highly dependent upon the type of starting material. Due to variations in consistency and viscosity, even similar sample types may require distinct handling.

The table below describes the recommendations in processing the primary samples before nucleic acid extraction:

**Table: Preparation of sample material for bacterial nucleic acid extraction**

| Sample type | Procedure |
|---|---|
| Inactivation the pathogenic microorganism | Recommended pre-treatment: Boiling. Incubate samples at 95°C for 10 min. Centrifuge briefly to collect the complete sample volume at the bottom of the tube. Allow samples to cool down or chill on ice, then proceeding the following steps according to the sample type |
| Viscous samples e.g. BAL, sputum or other mucous specimen | Recommended pre-treatment: Liquefaction. Prepare a fresh DTT stock solution for liquefaction* (e.g., 5× conc. DTT stock is about 0.75%). Adjust the final DTT concentration in the sample to0.15% by adding DTT stock solution. Incubate the sample (e.g., with shaking at 850 RPM for 30 min at 37°C) until it can be pipette easily. Transfer 200 µl to sample tube (Supplied in the kit) |
| For large volume liquid samples that have low or unknown bacterial loads e.g. urine, water collected from pool/river stream/tower | Recommended pre-treatment: Centrifugation. Centrifuge the sample for up to 10 min at 20000 × g to concentrate the bacterial cells in pellet. Discard supernatant, resuspend the pellet in 220 µl PBS. Take 200 µl to sample tube (Supplied in the kit) |
| Swab samples, e.g. eye, nasal, pharyngeal, or other swabs | Collect samples and place in 1 ml PBS containing a common fungicide. Incubate for 30min at room temperature. Take 200 µl to sample tube |
| For some gram-positive bacterial species. Especially for samples that contain particles, e.g. stool | Recommended pre-treatment : Mechanical homogenization<br>Follow the regular homogenization procedures in the laboratory. |
| Bacterial suspension cultures | Take 200 µl culture to sample tube |
| Bacterial colony | Take 1-3 bacterial colony from culture plate with an inoculation loop and suspend in 220 µl PBS by vigorous stirring. Take 200 µl suspension to sample tube |

### 15.9. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 15.10. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Viral/Pathogen Nucleic acids Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

* See Controls / internal control below

** The liquefaction could be done by using other solutions, such as NALC(N-Acetyl-L-Cysteine)-NaOH or other agents which could digest mucous material

# 16. BioMagPure Viral RNA Extraction kit

Cat. No. BS-060201-NK
Process time: BioMagPure 12S – 40-50 minutes
BioMagPure 24 – 40-55 minutes

### 16.1. Intended use

BioMagPure Viral Nucleic RNA Extraction Kit is used with the BioMagPure instrument for extraction of Viral RNA from human biological specimens such as serum, plasma, and other cell-free fluids.

### 16.2. Application

Nucleic acids extracted from BioMagPure Viral Nucleic Acid Extraction kit can be used in a number of downstream application including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, Southern Blot Analysis

### 16.3. Number of tests

48 extractions

### 16.4. Kit components

| Kit Contents | BS-060201-NK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter tip | 50 pcs. (50x1) |
| Piercing Pin | 50 pcs. (50x1) |
| Sample Tube (2 mL) | 50 pcs. (50x1) |
| Elute Tube (1.5 mL) | 50 pcs. (50x1) |
| RNA Carrier (1mg) | 1 pce. |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 16.5. Reagent cartridge contents

**Cell 1** **Cell 2** **Cell 3** **Cell 4** **Cell 5** **Cell 6** **Cell 7** **Cell 8** **Cell 9** **Cell 10**

| | | |
|---|---|---|
| Cell-1 | Proteinase K solution | 30 µl |
| Cell-2 | Lysis Buffer 4 | 720 µl |
| Cell-3 | Binding Buffer 1 | 1000 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 2 | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | RNase-free water | 1000 µl |
| Cell-9 | RNase-free water | 1000 µl |
| Cell-10 | Empty | |

### 16.6. Storage

BioMagPure Viral RNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition.

After dissolve the RNA carrier, store it at 4°C (short-term, up to 1 month) or -20°C (long-term). Do not freeze–thaw the Frozen RNA carrier more than3 times.

Store the nucleic acid at 4°C (up to 24hours) or -20°C for longer storage. Repeated freeze–thawing is not allowed.

### 16.7. Starting material

| Sample Type | Target Nucleic Acid | Sample Volume (Amount of starting material) | Elution Volume |
|---|---|---|---|
| Serum | Total Viral Nucleic Acids (DNA + RNA) | 100-400 µl | 50-300 µl |
| Plasma | | | |
| CSF | | | |
| Pre-treated Urine | | | |
| Cell-free body fluids | | | |
| Controls / internal control* | Add controls /internal control in the extraction procedure if the downstream analysis needed | | |

The kit is designed for extraction of viral RNA from plasma or serum, or from a pool of such cell-free body fluids. After extraction, store the nucleic acid at 4°C (up to 1 hour) or -20°C for longer storage. Repeated freeze–thawing is not allowed.

### 16.8. Sample preparation

The purification procedure is optimized for use with 100-400 µl serum, plasma, CSF, or pre-treated urine samples. (Blood samples treated with EDTA or citrate as an anticoagulant can be used for plasma preparation)

Samples can be either fresh or frozen, provided that they have not been refrozen after thawing

After collection and centrifugation, plasma, serum, or CSF can be stored at 2–8°C for up to 6 hours. For longer storage, we recommend freezing aliquots at –20°C or –80°C. Thaw samples at room temperature (15–25°C), and process the samples immediately when they have equilibrated to room temperature. Do not refreeze the aliquots after thawing. Repeated freeze–thawing leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids. If cryoprecipitates are visible in the samples, centrifuge at 6800 x g for 3 minutes, transfer the supernatants to fresh tubes without disturbing the pellets, and start the purification procedure immediately.

### 16.9. RNA carrier

For RNA virus, adding RNA carrier to the sample before extraction is recommended!

Add 1 ml RNase free water to the RNA carrier tube (provided with the kit) and mix by vortexing. Store it at 4°C (short-term, up to 1 month) or -20°C (long-term). Do not freeze–thaw the Frozen RNA carrier more than three times.

Add 5 µl RNA carrier (for 100 µl sample), 10 µl (for 200 µl sample) or 20 µl (for 400 µl sample) into the sample tube before add sample.

### 16.10. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 16.11. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Viral RNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

* See Controls / internal control below

# 17. BioMagPure Plant DNA Extraction Kit

Cat. No. BS-060201-OK
Process time: BioMagPure 12S – 45-55 minutes
BioMagPure 24 – 45-60 minutes

### 17.1. Intended use

BioMagPure Plant DNA Extraction Kit is used with the BioMagPure instrument for extraction of genomic DNA from plant (leaf, seeds and spores) and fungal tissues. Up to 100 mg of tissue can be used for purification.

### 17.2. Application

Nucleic acids extracted from BioMagPure Plant DNA Extraction kit can be used in a number of downstream application including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, Southern Blot Analysis.

### 17.3. Number of tests

48 extractions

### 17.4. Kit components

| Kit Contents | BS-060201-OK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter Tip | 50 pcs. |
| Piercing Pin | 50 pcs. |
| Sample Tube (2 mL) | 50 pcs. |
| Elute Tube (1.5 mL) | 50 pcs. |
| Filter Column | 50 pcs. |
| Collection Tube | 50 pcs. |
| RNase A (10mg/mL) | 1 pce. (0.5 ml) |
| Buffer PLA (25mL) | 1 pce. |
| Buffer PLB (25mL) | 1 pce. |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 17.5. Reagent cartridge contents

**Cell 1** **Cell 2** **Cell 3** **Cell 4** **Cell 5** **Cell 6** **Cell 7** **Cell 8** **Cell 9** **Cell 10**

| | | |
|---|---|---|
| Cell-1 | Empty | |
| Cell-2 | Lysis Buffer 2 | 720 µl |
| Cell-3 | Binding Buffer 1 | 720 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 1B | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | Elution Buffer 1 | 1000 µl |
| Cell-9 | Elution Buffer 2 | 1000 µl |
| Cell-10 | Empty | |

### 17.6. Storage

BioMagPure Plant DNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition

Store the purified DNA at 4 °C (short-term) or aliquot and store at -70°C (long-term) before perform the downstream analysis.

### 17.7. Starting material

After harvesting plant tissues, it should be frozen in liquid nitrogen if not be used immediately. It can then be stored at –80°C.Alternatively; tissue can be dried or lyophilized after harvesting to allow storage at room temperature (15–25°C). To ensure DNA quality, samples should be completely dried within 24 hours of collection

If possible, it is preferable to collect young materials (e.g., leaves, needles) since they contain more cells per weight and therefore result in higher yields

When working with fungi, harvest mycelium directly from a culture dish or from liquid culture. For liquid culture, first pellet cells by centrifugation. Remove the supernatant completely before disruption and lysis. Fresh, frozen, or freeze-dried fungal material can be use

The disruption method may require optimization to ensure maximum DNA yield and quality. Complete and quick disruption of starting material is essential to ensure high DNA yields and to avoid DNA degradation

Before DNA extraction, plant material is first mechanically disrupted with lysis buffer (Buffer PLA or PLB). After Homogenization, remove the debris and other precipitations by spin through the filter membrane. Collect the clear flow-through and incubate with RNase A to digest the RNA in the sample before DNA extraction.

### 17.8. Sample preparation

Using homogenizer to treat the tissue before extraction is recommended.

For better DNA yield, adding RNase A to sample before extraction processing.

| Sample type | Procedure |
|---|---|
| Plant tissue | Perform homogenization by using proper homogenizer<br>Add 440 µl plant lysis buffer* to sample<br>Vortex vigorously<br>Incubate the mixture at 65°C, 10min in a thermomixer (set at 1000 RPM) or vortex several times during incubation in the heat block or water bath.<br>Remove Lysate to filter membrane sitting in collection tube<br>Short spin at 6000g to collect clear flow-through<br>Add 10 µl RNase A, mix well, incubate for 10min at room temperature<br>Transfer to sample tube<br>Perform extraction |
| Yeast | Suspension culture<br>Centrifuge at 6000g, 3min<br>Remove supernatant<br>Add 440mL plant lysis buffer*, vortex mixing 30 s<br>Incubate the mixture at 65°C, 10min in a thermomixer (set at 1000 RPM) or vortex several times during incubation in the heat block or water bath.<br>Remove Lysate to filter membrane sitting in collection tube<br>Short spin at 6000g to collect clear flow-through<br>Transfer 400 µl to sample tube<br>Perform extraction<br><br>Culture colony<br>Take 1-3 colony from culture plate with an inoculation loop and suspend in 440 µl of plant lysis buffer* by vigorous stirring<br>Incubate the mixture at 65°C, 10min in a thermomixer (set at 1000 RPM) or vortex several times during incubation in the heat block or water bath.<br>Remove Lysate to filter membrane sitting in collection tube<br>Short spin at 6000g to collect clear flow-through<br>Take 400 µl suspension to sample tube<br>Perform extraction |

* We provide two kind plant lysis buffer: PLA and PLB for dealing with different tissue types. Before extraction a new tissue type, try these two plant lysis buffer for getting the optimized lysis procedure and a better DNA yield. If the precipitation formed in the lysis buffer, warm it at 65°C before use

### 17.9. Result

| Plant | Tissue type | Con. (ng/ µl) |
|---|---|---|
| Soybean-100mg | Seed | 5-12 (PLA) / 50-80 (PLB) |
| Rice-20mg | Seed | 5-8 (PLA) / 15-25 (PLB) |
| Arabidopsis-100mg | leaf | 2-5 (PLA) / 5-7 (PLB) |
| Tomato-100mg | leaf | 20-40 (PLA) |
| Corn-100mg | leaf | 10-15 (PLA) / 25-60 (PLB) |
| Tectaria-100mg | leaf | 5-10 (PLA) |
| Aspidistra-100mg | leaf | 3-6 (PLA) |
| Pharius-100mg | leaf | 20-25 (PLA) / 50-100 (PLB) |
| Zingiber-100mg | leaf | 3-8 (PLA) / 20-25 (PLB) |

### 17.10. Controls / internal controls

Use appropriate controls for downstream analysis:

| **Type** | **Description** | **Location** |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 17.11. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Plant DNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

# 18. BioMagPure Total RNA Extraction Kit

Cat. No. BS-060201-PK
Process time: BioMagPure 12S – 35-45 minutes
BioMagPure 24 – 35-50 minutes

### 18.1. Intended use

BioMagPure Total RNA Extraction Kit is used with the BioMagPure instrument for extraction of total RNA from whole blood, blood cells, animal tissue, plant tissue, yeast or cultured cells.

### 18.2. Application

Total RNA extracted from BioMagPure Total RNA Extraction kit can be used in a number of downstream application including: RT-PCR, qPCR, Sequencing (NGS), Microarray, Northern blotting

### 18.3. Number of tests

48 extractions

### 18.4. Kit components

| Kit Contents | BS-060201-PK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter Tip | 50 pcs. |
| Piercing Pin | 50 pcs. |
| Sample Tube (2 mL) | 50 pcs. |
| Elute Tube (1.5 mL) | 50 pcs. |
| RL A Buffer (25 mL) | 1 pce. |
| RL B Buffer (25 mL) | 1 pce. |
| Filter column | 50 pcs. |
| Collection tube | 50 pcs. |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 18.5. Reagent cartridge contents

**Cell 1** **Cell 2** **Cell 3** **Cell 4** **Cell 5** **Cell 6** **Cell 7** **Cell 8** **Cell 9** **Cell 10**

| | | |
|---|---|---|
| Cell-1 | Empty | 30 µl |
| Cell-2 | Lysis Buffer4 | 720 µl |
| Cell-3 | Binding Buffer 1 | 1000 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 2 | 1000 µl |
| Cell-6 | Washing Buffer A | 1000 µl |
| Cell-7 | Washing Buffer B | 1000 µl |
| Cell-8 | RNase-free water | 1000 µl |
| Cell-9 | RNase-free water | 1000 µl |
| Cell-10 | Empty | |

### 18.6. Storage

BioMagPure Total RNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition

After extraction, store RNA at -60 to -80°C immediately, repeated freeze–thawing is not allowed. Always handle RNA on ice for downstream analysis

Store the RLA and RLB in 4°C when received the kit.

### 18.7. Purification protocol

| Protocol name | Sample vol.<br>Elute vol. | Description |
|---|---|---|
| Total RNA | 100-400 µl<br>50-200 µl | Extraction Total RNA and DNA from sample |
| Total RNA (DNA-free) | 100-400 µl<br>50-200 µl | Extraction total RNA (DNA-free)* from sample |

### 18.8. Before starting

β-Mercaptoethanol (β-ME) must be added to Buffer RLA and RLB before use (store at 4°C for 4 months)

If performing DNA-free protocol, Prepare DNase before extraction. Place 10 µl DNase in the first elute product. Homogenization is necessary for animal tissue, Plant tissue and yeast before extraction. Add RL Buffers to sample when perform homogenizing. Wear clean glove, use RNase-free filter tip, and keep work area, pipettors and reagents free of virus, bacteria and Nuclease contamination. Using RNase Zap® to clean the surface of bench, equipment and pipettors is one of the easiest way to remove the RNase contaminations of work area. Using RNA stabilized reagent (e.g. RNA later) to treat sample is one of the best way to protect the RNA if the sample cannot be processing in an RNase-free working area.

### 18.9. Reagents to be supplied by user

| Reagent | Description | Preparation |
|---|---|---|
| β-mercaptoethanol (β-ME) | β-ME reduce disulfide bonds and irreversibly denature the RNase and eliminate RNase released during cell lysis | Add 10 µl β-ME per 1 ml RL lysis Buffers*. It can be stored at RT for up to one month |
| Red blood cells lysis buffer (RBC lysis buffer) | Lyse Erythrocyte from whole blood (Erythrocyte (RBC) lysis procedure) | 10xRBC lysis buffer (100ml).<br>8.29g $NH_4Cl$ (1.5M)<br>1g $KHCO_3$ (100mM)<br>0.0372g $Na_2EDTA$ (10mM)<br>Adjust pH7.2-7.4 by HCl<br>0.2 mm filtered, store for 6 months at 4℃<br>Dilute 10 times fresh before use |
| DNase | To eliminate DNA contamination | Novagen RNase-free DNase I (69182-3CN) |
| 10x DNase buffer | To eliminate DNA contamination | 0.5M Tris-HCl<br>25mM $MgCl_2$<br>5mM $CaCl_2$ |

### 18.10. Starting material

#### 18.10.1. Whole blood

Using the fresh whole blood sample for isolation. (within 4 hr, on ice) Freezing blood is not allowed. The blood sample should be collected in the presence of an anticoagulant, preferably EDTA, although other anticoagulants such as citrate, heparin, or ACD (acid citrate dextrose) can also be used.

For optimal results, blood samples should be processed within a few hours of collection and keep in 4℃.

Performing Erythrocyte (RBC) lysis procedure before extraction.

Using the whole blood samples which have extreme high WBCs no. (more than 10000/ µl) or concentrated PBMCs (peripheral blood mononucleated cells), decrease the input volume for extraction is recommended (total WBC no. less than $5 \times 10^6$).

#### 18.10.2. Tissue

To prevent degradation by intracellular RNase, it is important that tissues are either flash-frozen in liquid nitrogen or stored at –70°C, or processed immediately following excision.

Using RNA stabilized reagent (e.g. RNA later) to treat tissue is another option to protect the RNA if the sample cannot be froze immediately. Frozen tissue should not be allowed to thaw during handling (e.g., weighing), keep sample on ice during cutting or homogenized with RLA Buffer is recommended.

After homogenization, using filter column (supplied in the kit) to remove the insoluble and viscous material of the lysates.

#### 18.10.3. Cells

Cells or isolated blood cells can be collected as pellets and either flash-frozen in liquid nitrogen and stored at –70°C, or processed immediately. Add RLA Buffer to resuspend pellet for extraction. Alternatively, samples can be stored at –70°C in RLA Buffer after disruption and homogenization. Samples frozen in this way are stable for months.

* For Total RNA (DNA-free), preparation of DNase (not supplied in the kit) is necessary

### 18.10.4. Plant tissue and yeast

Up to 100 mg of sample is first ground in liquid nitrogen or frozen, then add lysis buffer (RLA or RLB Buffer) to homogenizer

Most Plant cells use RLA Buffer for disruption and denaturing sample

However, some tissues, such as milky endosperm of maize or mycelia of filamentous fungi, solidify in RLA Buffer, making the extraction of RNA impossible. In these cases, RLB Buffer should be used instead.

After add lysis buffer (RLA or RLB), samples are place into homogenizer for homogenization

After homogenization, using filter column (supplied in the kit) to remove the insoluble and viscous material of the lysates.

It is essential to use the correct amount of starting material in order to obtain optimal RNA yield and purity (as the table below). Use excess quantity is not helpful in total RNA extraction.

| Sample Type | Sample Volume (Amount of starting material) | Elution Volume |
|---|---|---|
| Whole blood | 200-400 µl* (WBCs no. about 106) | 50-200 µl** |
| PBMCs | Up to 50 µl (suspended in 200 µl with RL buffer) | |
| Tissue | 10-40mg (Lysed and suspend with RL buffer) | |
| Cultured cells | 200-400 µl suspension of primary or cultured cells (cell no. < 5 x 106) | |
| Plant tissue | Up to 100mg | |
| Yeast | Up to 100mg | |
| Controls/internal control | Add controls /internal control in the extraction procedure if the downstream analysis needed | |

### 18.11. Sample preparation

| Sample | Procedure |
|---|---|
| Whole blood | Fresh prepare 1x RBC lysis buffer. Add ice-cold two volume RBC lysis buffer to one volume blood sample. Inverting 3-5 times, incubate on ice for 10-15 min. Centrifuge at 1000 g, 10min, and 4 ˚C. Remove supernatant. Resuspend pellet with 220 µl RLA Buffer. Take 200 µl for extraction |
| PBMCs (Peripheral Blood Mononucleated Cells) | Resuspend PBMCs with 220 µl RLA Buffer. Vortex mixing for 10 sec. Take 200 µl for extraction |
| Tissue | Add 220 µl RLA Buffer to tissue; make sure the sample if completely immersed in buffer. Increase RLA buffer input amount up to 440 µl if tissue sample is large. Homogenized tissue by homogenizer. Spin down the lysate. Remove all the lysate to filter column sitting in collection tube. Centrifuge at 1000 g, for 5min on 4 ˚C. Transfer 200-400 µl to sample tube. Perform extraction |
| Cultured cells | Protocol 1. Suspension culture.<br>Harvest cell culture. Centrifuge at 1000xg, 5min on 4 ˚C. Remove supernatant completely. Resuspend cell pellet with 220 µl RLA Buffer. Vortex mixing for 10 sec. Take 200 µl for extraction |
| | Protocol 2-1. Monolayer culture.<br>Trypsinize the cells. Harvest the cell in PBS. Centrifuge at 300xg, 5min on 4 ˚C. Remove supernatant. Resuspend pellet with 220 µl RLA Buffer. Vortex mixing for 10sec. Take 200 µl for extraction. |
| | Protocol 2-2. Monolayer culture.<br>Scrape the cells with 220-440 µl RLA Buffer. Vortex mixing for 10 sec. Take 200-400 µl for extraction |
| Plant tissue/ Yeast | Add 220 -440 µl RLA or RLB Buffer to sample, make sure the sample if complete immersed in buffer. Homogenized tissue by homogenizer. Remove lysate to filter column sitting in collection tube. Centrifuge at 1000 x g, for 5min on 4 ˚C. Spin down. Transfer 200-400 µl to sample tube. Perform extraction |
| DNA-free RNA extraction | After total RNA program extraction. Add 2 µl DNase in the eluate. Incubate at 37 ˚C, 10min. Transfer mixture to a new sample tube. Proceeding “Total RNA” protocol to start extraction |

### 18.12. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 18.13. Quality control

In accordance with BIOSAN’s ISO-certified Quality Management System, each lot of BioMagPure Total RNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

* Blood cells needs to perform manual RBC lysis procedure before extraction

** After extraction, store RNA at -60 to -80°C immediately, repeated freeze–thawing is not allowed

# 19. BioMagPure Viral Nucleic Acid Large Volume Extraction Kit

Cat. No. BS-060201-QK
Process time: BioMagPure 12S – 60-95 minutes
BioMagPure 24 – 60-100 minutes

### 19.1. Intended use

BioMagPure Viral Nucleic Acid Large Volume Extraction Kit is used with the BioMagPure instrument for extraction of Viral DNA or RNA from human biological specimens such as serum, plasma, and other cell-free fluids.

### 19.2. Application

Nucleic acids extracted from BioMagPure Viral Nucleic Acid Large Volume Extraction kit can be used in a number of downstream applications including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, and Southern Blot Analysis.

### 19.3. Number of tests

48 extractions

### 19.4. Kit components

| Kit Contents | BS-060201-QK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter Tip | 50 pcs. |
| Piercing Pin | 50 pcs. |
| Sample Tube (2 mL) | 50 pcs. |
| Elute Tube (1.5 mL) | 50 pcs. |
| RNA Carrier (1mg) | 2 pcs. |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 19.5. Reagent cartridge contents

**Cell 1** **Cell 2** **Cell 3** **Cell 4** **Cell 5** **Cell 6** **Cell 7** **Cell 8** **Cell 9** **Cell 10**

| | | |
|---|---|---|
| Cell-1 | Proteinase K solution | 30 µl |
| Cell-2 | Lysis Buffer 4 | 1100 µl |
| Cell-3 | Binding Buffer 1 | 1600 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Washing Buffer 2 | 1100 µl |
| Cell-6 | Washing Buffer A | 1100 µl |
| Cell-7 | Washing Buffer B | 1100 µl |
| Cell-8 | RNase-free water | 1000 µl |
| Cell-9 | RNase-free water | 1000 µl |
| Cell-10 | Empty | |

### 19.6. Storage

BioMagPure Viral Nucleic Acid Large Volume Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition.

After dissolve the RNA carrier, store it at 4°C (short-term, up to 1 month) or -20°C (long-term). Do not freeze–thaw the Frozen RNA carrier more than3 times.

Store the nucleic acid at 4°C (up to 24hours) or -20°C for longer storage. Repeated freeze–thawing is not allowed.

### 19.7. Starting material

| Sample Type | Target Nucleic Acid | Sample Volume (Amount of starting material) | Elution Volume |
|---|---|---|---|
| Serum | Total Viral Nucleic Acids (DNA + RNA) | 400-1000 µl | 50-300 µl |
| Plasma | | | |
| CSF | | | |
| Pre-treated Urine | | | |
| Cell-free body fluids | | | |
| Controls/internal control* | Add controls /internal control in the extraction procedure if the downstream analysis needed | | |

The kit is designed for extraction of viral nucleic acids (e.g., those of HIV, HCV, HBV, or HPV) from plasma or serum, or from a pool of such cell-free body fluids

After extraction, store the nucleic acid at 4°C (up to 24hours) or -20°C for longer storage. Repeated freeze–thawing is not allowed

### 19.8. Sample preparation

The purification procedure is optimized for use with 400-1000 µl serum, plasma, CSF, or pre-treated urine samples. (Blood samples treated with EDTA or citrate as an anticoagulant can be used for plasma preparation)

Samples can be either fresh or frozen, provided that they have not been refrozen after thawing

After collection and centrifugation, plasma, serum, or CSF can be stored at 2–8°C for up to 6 hours. For longer storage, we recommend freezing aliquots at –20°C or –80°C. Thaw samples at room temperature (15–25°C), and process the samples immediately when they have equilibrated to room temperature. Do not refreeze the aliquots after thawing. Repeated freeze–thawing leads to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral nucleic acids. If cryoprecipitates are visible in the samples, centrifuge at 6800 x g for 3 minutes, transfer the supernatants to fresh tubes without disturbing the pellets, and start the purification procedure immediately.

### 19.9. RNA carrier

For RNA virus, adding RNA carrier to the sample before extraction is recommended!

Add 1.0 ml RNase free water to the RNA carrier tube (provided with the kit) and mix by vortexing. Store it at 4°C (short-term, up to 1 month) or -20°C (long-term). Do not freeze–thaw the Frozen RNA carrier more than3 times

Add 20-40 µl RNA carrier (for 400-1000 µl sample) into the Sample Tube.

### 19.10. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 19.11. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Viral Nucleic Acid Large Volume Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

* See Controls / internal control below

# 20. BioMagPure CFC DNA Extraction Kit LV

Cat. No. BS-060201-RK
Process time: BioMagPure 12S – 90-100 minutes
BioMagPure 24 – 90-100 minutes

### 20.1. Intended use

BioMagPure CFC DNA Extraction Kit LV provide fast, reliable and simple procedures for isolating cell-free circulating DNA (cfc-DNA) from 2.0 ml plasma/serum samples using BioMagPure automated nucleic acid extraction systems. Purification is based on magnetic bead separation that uses Biosan proprietary BioMagPure bead separation technology. The kits are designed to isolate all sizes of cfc-DNA from either fresh or frozen plasma/serum samples.

### 20.2. Application

Nucleic acids extracted from BioMagPure CFC DNA Extraction kit LV can be used in a number of downstream applications including: PCR, qPCR, Sequencing (NGS), Microarray, RFLP, and Southern Blot Analysis.

### 20.3. Number of tests

48 extractions

### 20.4. Kit components

| Kit Contents | BS-060201-RK-48 |
|---|---|
| Reagent Cartridge | 48 pcs. (6x8) |
| Reaction Chamber | 48 pcs. (6x8) |
| Tip Holder | 48 pcs. (6x8) |
| Filter Tip | 50 pcs. |
| Piercing Pin | 50 pcs. |
| Sample Tube (7 mL) | 50 pcs. |
| Elute Tube (1.5 mL) | 50 pcs. |
| Small tip | 50 pcs. |
| RLA Buffer (100 mL) | 1 pce. |
| Proteinase K (10 mL) | 1 pce. |
| Barcode Paper | 1 pce. |
| Selection guide | 1 pce. |

### 20.5. Reagent cartridge contents

**Cell 1** **Cell 2** **Cell 3** **Cell 4** **Cell 5** **Cell 6** **Cell 7** **Cell 8** **Cell 9** **Cell 10**

| | | |
|---|---|---|
| Cell-1 | Empty | |
| Cell-2 | Washing Buffer 5 | 800 µl |
| Cell-3 | Washing Buffer 2A | 800 µl |
| Cell-4 | Magnetic Bead Solution | 800 µl |
| Cell-5 | Binding Buffer 2 | 750 µl |
| Cell-6 | Binding Buffer 2 | 750 µl |
| Cell-7 | Binding Buffer 2 | 750 µl |
| Cell-8 | Binding Buffer 2 | 750 µl |
| Cell-9 | Elution Buffer | 800 µl |
| Cell-10 | Empty | |

### 20.6. Storage

BioMagPure CFC DNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 18 months under the condition.

Store the nucleic acid at 4°C (up to 24hours) or -20°C for longer storage. Repeated freeze–thawing is not allowed.

### 20.7. Starting material

| Sample Type | Target Nucleic Acid | Sample Volume (Amount of starting material) | Elution Volume |
|---|---|---|---|
| Serum | CFC DNA | 3.75 ml (2.0 ml sample + 1.6 ml RLA + 150 µl proteinase K) | 50-100 µl |
| Plasma | | | |
| Cell-free body fluids | | | |
| Controls/internal control * | Add controls /internal control in the extraction procedure if the downstream analysis needed | | |

The kit is designed for extraction of CFC DNA from plasma or serum, or from a pool of such cell-free body fluids. After extraction, store the nucleic acid at 4°C (up to 24hours) or -20°C for longer storage. Repeated freeze–thawing is not allowed.

### 20.8. Sample preparation

Transfer 2.0 ml of plasma, serum or cell-free body fluids into 7 ml sample tube.
Add 150 µl proteinase K into the tube
Add 1.6 ml RLA buffer into the tube
Close the cap and mix by pulse-vortexing for 30 seconds.
Incubate at 56°C for 30 minutes.
Start the extraction with BioMagPure system.

### 20.9. Controls / internal controls

Use appropriate controls for downstream analysis:

| Type | Description | Location |
|---|---|---|
| Positive control | Using sample which positive for target | Place in sample tube |
| Negative control | Using sample which negative for target or water(NTC) | Place in sample tube |
| Internal control(IC) | Using a defined quantity control | Place in sample tube or the round well of the reaction chamber |

### 20.10. Quality control

In accordance with BIOSAN's ISO-certified Quality Management System, each lot of BioMagPure Blood DN CFC DNA Extraction Kits is tested against predetermined specifications to ensure consistent product quality.

* See Controls / internal control below

# 21. Protocol of extraction

21.1. Turn the power switch on and waiting for the LCM screen turn on and shows “BioMagPure System Stand-By”.

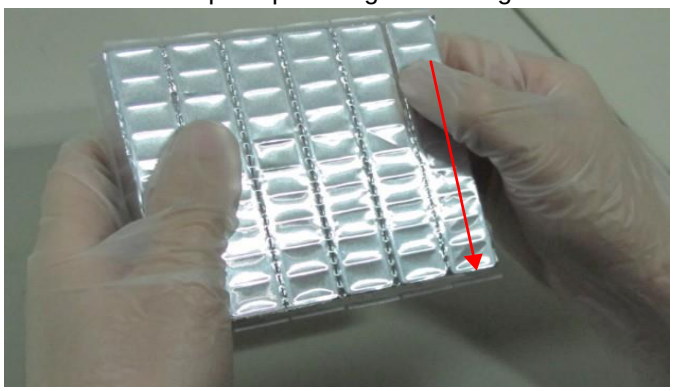
21.2. Press “Start” button. The system will process self-testing, and then go to steady mode.
**Note**: The system will block main functions before the completion of self-testing process.

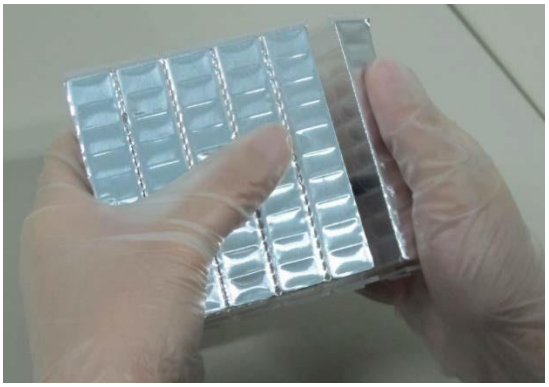
21.3. Open the sliding door and remove the sample rack from the instrument.

21.4. Load Reagent Cartridges, and all plastics disposables (Reaction Chamber, Tip Holder, Piercing Pin, Filter tip and Pestle (optionally supplied with some kit types)

**Insert the cartridges.**

21.5. How to pull apart reagent cartridges





**Slash open the dotted line with nail and snap it with a little bit force.**

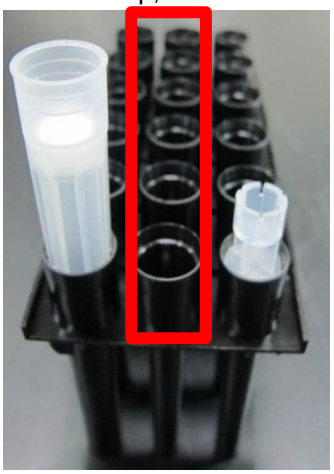
**Insert reaction chambers.**

**Insert tip holder.**

**Insert piercing pins.**

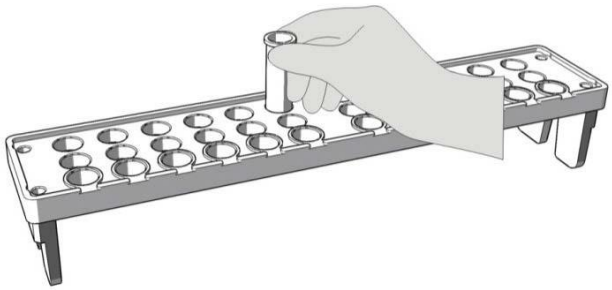
**Insert filter tips.**

**Note:** The positions of piercing pin and filter tip; the 2nd one should be empty.

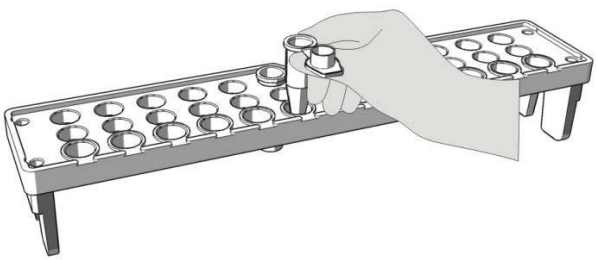


21.6. Load one Reagent Cartridge and one set of plastic disposable per sample.
**Important:**
Set Cartridges in the order of the number from left to right.
Make sure that Cartridges are inserted in to the Cartridge Tray tightly.
You can load 1-12 cartridges on the tray depending on the number of samples that you wish to process.

21.7. Load Sample Tube and Elute Tube to Sample Rack on the bench.

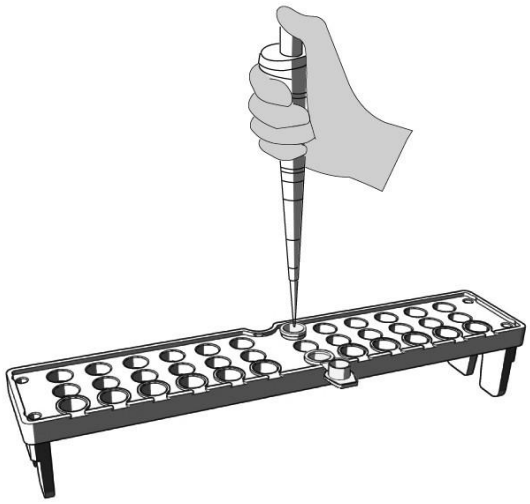


**Insert Sample Tube on to Sample Rack.**



**Insert Elute Tube on to Sample Rack.**

21.8. Load the sample (s) to Sample Tube

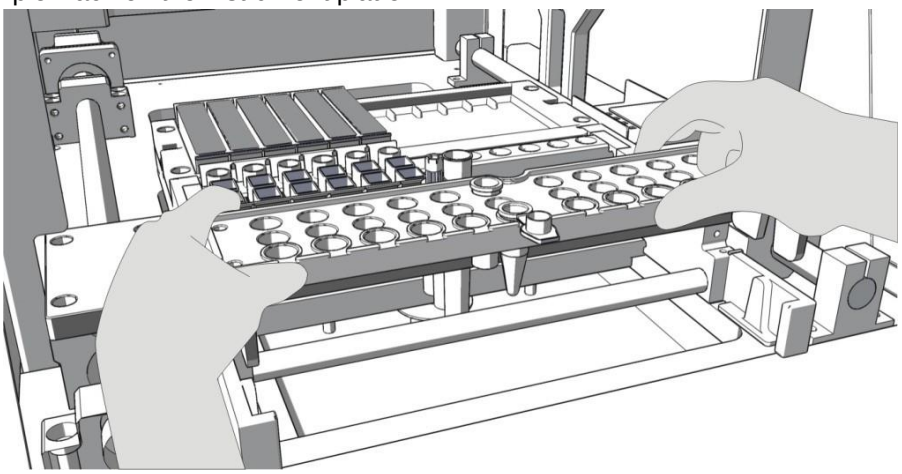


**Note:**

Pre-treatments are essential for some sample types before loading to Sample Tube. Please refer to the handbook of reagent kits for details.

Make sure the caps of Elute Tube are open as the figure shown above.

21.9. Place Sample Rack on the instrument platform



**Note:**

Use two hands to handle the Sample Tray.

Make sure the Sample Tray be placed correctly on to the instrument.

21.10. Close the door

21.11. Scan the protocol barcodes to select purification protocol, sample volume and elute volume.


**Note:**

There is one protocol barcode paper enclosed in the reagent kit box

Protocol's name, sample volume and elution volume will be shown on LCM screen after protocol barcode is scanned.

21.12. Follow the instructions displayed on LCM screen to double check the operating steps being completed before program running.

21.13. Push “Enter” to confirm. Instrument will start to run the protocol program automatically until whole processes are completed.
**Note:** It takes from 30 to 45 minutes to complete the extraction according to reagent types

21.14. At the end of the run, the instrument beeps briefly and the LCM shows “Protocol Completed”

21.15. Open the instrument door

21.16. Remove the elute tubes containing the purified nucleic acid
**Note:** Store the purified nucleic acids at 4°C for short-term storage or store at -70°C for long-term storage

21.17. Discard the used cartridges, all plastic consumables into biohazard waste. Do not reuse the cartridges

21.18. If you’re not using the instrument, place the Sample Rack back to workplace, close the instrument door and push “Start” button for 2 secs to get into “sleeping mode”.

21.19. And for longer time not using the instrument turn the power switch off.

**Biosan SIA**
Ratsupites 7, build.2, Riga, LV-1067, Latvia
Phone: +371 67426137 Fax: +371 67428101
**http://www.biosan.lv**