Reagent Kit Selection Guide

Reagent Kit Selection Guide (From Target Nucleic Acids)

Target	Type	Origin	Scale	Reagent Kits
DNA	Total DNA	Human, animal blood (fresh, old, dried, frozen whole blood with common anticoagulants, Buffy Coat	100-400 µl whole blood	AnaPrep Blood DNA Extraction Kit 200
			400-1000 µl whole blood	AnaPrep Blood DNA Extraction Kit 1200 *especially for the granulocytes-rich blood samples (white blood cell no. more than $2x10^4$ cells/µl)
DNA	Virus	Whole Blood	100-400 µl whole blood	AnaPrep Blood DNA Extraction Kit 200
DNA/RNA	Virus	Cell culture supernant, human serum, plasma, urine, cerebrospinal fluid, and other cell-free body fluids	See Reagent Handbook	AnaPrep Viral Nucleic Acid Extraction Kit
DNA	Virus/ Bacteria	Genital tract specimen (collected by cervical brush or genital swab), cervicovagina lavage, urine specimens	See Reagent Handbook	AnaPrep HPV DNA Extraction kit for swab samples
DNA	Total DNA	• Human and animal tissue (fresh and frozen tissues), Rodent tails • Insects (fresh and frozen tissue) • Dried blood • Dried Swab Material (buccal, nasal, pharyngeal, vaginal, eye swab or saliva)	See Reagent Handbook	AnaPrep Tissue DNA Extraction Kit
DNA	Total DNA	FFPE (formalin fixed paraffin embedded) tissue sections	See Reagent Handbook	AnaPrep FFPE DNA Extraction Kit
DNA	Total DNA	Cell culture, plasma, serum, bone marrow, buffy coat (fresh or frozen serum/plasma, cells in adherent/suspension culture, lavage)	See Reagent Handbook	AnaPrep Cultured Cell DNA Extraction Kit
DNA	Bacteria	Bacteria species (from different kinds of starting materials), bacteria pellets, liquid transport media, swabs and urine, colony	See Reagent Handbook	AnaPrep Bacterial DNA Extraction Kit *Special item: AnaPrep TB DNA Extraction Kit
DNA	Total DNA	Forensic material (whole blood, clotted blood, bones, teeth, ancient bones, hair roots, forensic surface and contact swabs, saliva, chewing gum, cigarette butts, stamps, envelops, tissue, etc.)	See Reagent Handbook	AnaPrep Forensic DNA Extraction Kit

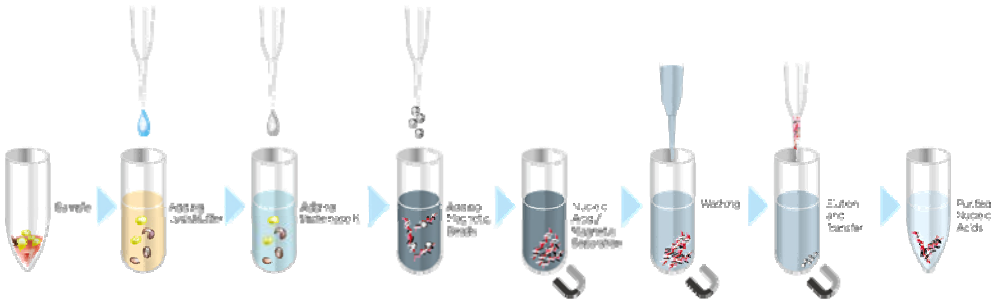
Introduction

The BioChain Nucleic Acid Preparation Technology

Introduction

BioChain Institute Inc. specializes in developing advanced, efficient and reliable technologies in nucleic acid preparation, to enable successful delivery of extraction results from varied sample types.

The AnaPrep technology is a state of the art platform that uses magnetic beads to extract nucleic acids from samples. The platform commits to a truly walk-away automation for nucleic acid purification from samples to results. The purification processes contain steps of lysis, binding, washing and elution (see figure below).



magnetic bead extraction process

Product information

Intended use

AnaPrep Kits are intended to be used with the AnaPrep 12 instrument for the preparation of nucleic acids from biological specimens. The AnaPrep instrument and AnaPrep reagent kits are not intended for use as part of a specific in vitro diagnostic test.

The nucleic acids purified using the AnaPrep 12 instrument and reagent kits are suitable for a variety of polymerase chain reaction (PCR) tests. The AnaPrep 12 instrument and reagent kits are intended for research use only.

Warranty

BioChain 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 services. If you have questions or concerns about our product or services, contact our Technical Support Representatives.

BioChain 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 BioChain Institute'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.

Satisfaction Guarantee	For any product that fails to perform satisfactorily due to any reason other than misuse, BioChain will replace it free of charge. Simply call BioChain or your distributor to get a replacement.
Technical Support	For technical assistance and more information, please visit our website at www.biochain.com or call the BioChain Technical Service Department or your local distributors.
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 (MSDS). You can find, download, view, and print them from our website www.biochain.com.
Manufacturer Information	**Manufacturer:** BioChain Institute Inc. **Address:** 39600 Eureka Dr. Newark, CA 94560, USA **Tel:** 1-510-783-8588 **Fax:** 1-510-783-5386 **Mail:** info@biochain.com **Country of Origin:** **USA**

AnaPrep Bacterial DNA Extraction Kit

Cat. No. Z1322006 Process Time: 60 minutes

Intended Use

The AnaPrep Bacterial DNA Extraction Kit is designed for use with the AnaPrep 12 or 24 instruments for extraction of genomic DNA from both Gram-positive and Gram-negative bacteria.

Application

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

Number Of Tests

48 extractions

Kit Components

Kit Contents	ZP1320010-48
Reagent Cartridge	48 pcs
Reaction Chamber	48 pcs
Tip Holder	48 pcs
Filtered Tip	50 pcs
Piercing Pin	50 pcs
Sample Tube (2 mL)	50 pcs
Elution Tube (1.5 mL)	50 pcs
Buffer BL2B (25mL)	1 pc
Barcode Paper	1 pc

Reagent Cartridge Content

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

Storage

- The AnaPrep Bacterial DNA Extraction Kit should be stored at room temperature (15-25°C). Do not freeze the reagent cartridges. The Kits are stable for 12 months under the proper storage conditions.
- Store the purified DNA at 4°C (short- term) or aliquot and store at -70°C (long-term).

Starting Material

- Bacterial pellet/colony from culture, cell-free body fluids, liquid transport media, urine, environment material (water, soil, etc.).
- If using paraffin-embedded tissue sections as samples, we recommend extracting DNA by using the AnaPrep FFPE DNA Extraction kit (Z1322009).
- If using tissue samples, we recommended using the AnaPrep Tissue DNA Extraction kit.
- The amount and type of starting material required for use in the AnaPrep 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	100-400 µl /Up to 10^9 bacteria (about OD_{600} = 3)	100-300 µl
Bacterial colony		100-400 µl /1-3 colony	
Tissue		100-400 µl /1-30 mg	
Urine		100-400 µl /5-50 ml urine	
Cell-free body fluids		100-400 µl cell-free body fluids	
Liquid transport media		100-400 µl liquid transport media	
NOTE: Before extraction, adjust sample volume with buffer BL2.			

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 BL2 is specialized for bacterial cell wall lysis*** (Supplied in the kit), use it to resuspend the bacterial pellet before processing extraction.
  * For *mycobacterium spp*. (e.g. MTB), use buffer BL3 for bacterial cell wall lysis (buffer BL3 is supplied in the Z1322008 AnaPrep TB DNA Extraction kit).

- 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 e.g. BAL, sputum or other mucous specimen	**Recommended pretreatment : Liquefaction** 1. Prepare a fresh DTT stock solution for liquefaction * (e.g., 5× conc. DTT stock is about 0.75%). 2. Adjust the final DTT concentration in the sample to 0.15% by adding DTT stock solution. 3. Incubate the sample (e.g., with shaking at 850 r.p.m. for 30 min at 37°C) until it can be pipetted easily. 4. Pellet bacteria by centrifugation at 14000 x g for 10 min.

	5. Discard the supernatant, and resuspend the pellet in 220 µl Buffer BL2. 6. Transfer 200 µl suspension to sample tube (Supplied in the kit). * 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.
For large volume liquid samples that have low or unknown bacterial loads e.g. urine, water collected from pool/river stream/tower	**Recommended pretreatment : Centrifugation** 1. Centrifuge the sample for up to 10 min at 20,000 × g to concentrate the bacterial cells into a pellet. 2. Discard supernatant and resuspend the pellet in 220 µl Buffer BL2* 3. Take 200 µl of the suspension and add it to the sample tube (supplied in the kit). * If there are visible particles in the pellet, centrifuge again after BL2 buffer treatment or filtering out the dust is recommended.
For cell-free body fluids (e.g. CSF, BAL, aspirates)	**Recommended pretreatment : Centrifugation** (Method 1) 1. Pellet bacteria by centrifugation at 14000 x g for 10 min. 2. Re-suspend the bacterial pellet in 220 µl Buffer BL2. 3. Take 200 µl of the suspension and add it to the sample tube (supplied in the kit). (Method 2-Centrifugation free) 1. Take 200 µl of the sample and add to a 1.5 ml centrifugation tube. 2. Add 200 µl of buffer BL2 to the sample (1:1). 3. Vortex-mix for 5-10 sec.

	4. Transfer 400 µl of the sample to the sample tube (supplied in the kit).
For swab samples e.g. eye, nasal, pharyngeal, or other swabs	(Method 1) 1. Collect samples and place in 2 ml PBS containing a common fungicide. Incubate for 30 min at room temperature. 2. Pellet bacteria by centrifugation at 14000 x g for 10 min. 3. Resuspend the bacterial pellet in 220 µl Buffer BL2 (supplied in the kit). 4. Take 200 µl of the suspension and add it to the sample tube (supplied in the kit). (Method 2: Centrifugation free) 1. Place the sample swab in 440 µl buffer BL2 and incubate for 30 min at room temperature. 2. Transfer 400 µl to the sample tube.
Enhance lysis efficiency of certain bacteria e.g. Gram-positive species	**Recommended pretreatment : Enzymatic Digestion** 1. Pellet bacteria by centrifugation at 14000 x g for 10 min. 2. Suspend bacterial pellet in 200 µl of the appropriate enzyme solution* 3. Incubate for at least 30 min at 37°C 4. Add 220 µl Buffer BL2. Mix by vortexing. 5. Take 200 µl suspension to sample tube (supplied in the kit). * enzyme solution : 20 mg/ml lysozyme or 200 µg/ml lysostaphin; 20 mM Tris-HCl, PH 8.0; 2 mM EDTA; 1.2% Triton)
For some gram-positive bacterial species, Especially for samples that contain particles e.g. stool	**Recommended pretreatment : Mechanical homogenization** ◆ Follow the regular homogenization procedures in the laboratory. ◆ For some sample types, DNA yield can be

	improved by performing the homogenization step before adding buffer BL2 and Proteinase K.
Isolation of genomic DNA from bacterial suspension cultures	1. Pipet 1 ml of the bacterial culture into a 1.5 ml microcentrifuge tube and centrifuge at 5000 x g for 5 min. 2. Discard the supernatant. 3. Add 220 µl Buffer BL2 to the pellet and vortex for 5-10 min. 4. Take 200 µl of the suspension and add it to the sample tube (supplied in the kit).
Isolation of genomic DNA from bacterial plate culture	1. Take 1-3 bacterial colonies from the culture plate with an inoculation loop and suspend in 220 µl of buffer BL2 by vigorous stirring. 2. Take 200 µl of the suspension and add to the sample tube (supplied in the kit).
To inactivate pathogenic organisms in the sample	**Recommended pretreatment : Boiling** 1. Incubate samples at 95°C for 10 min. 2. Centrifuge briefly to collect the complete sample volume at the bottom of the tube. 3. Allow samples to cool down or chill on ice, then transfer 100-400 µl of the cooled sample to the sample tube.

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.

Result

(1) Scalability

The AnaPrep Bacterial DNA Extraction kit was used to extract the DNA from cultured *Escherichia. coli* (ATCC25922) and *Staphylococcus aureus* (ATCC27154) in LB broth at different bacterial density (measure the Optical Density at 600nm; OD_{600}). Take 200 µl of the 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 analyzed by 1% TAE agarose gel electrophoresis (fig.1b and 2b). The results indicate that the nucleic acid extraction in both Gram-negative (*E.coli*) and Gram-positive (*S. aureus*) have excellent scalability.

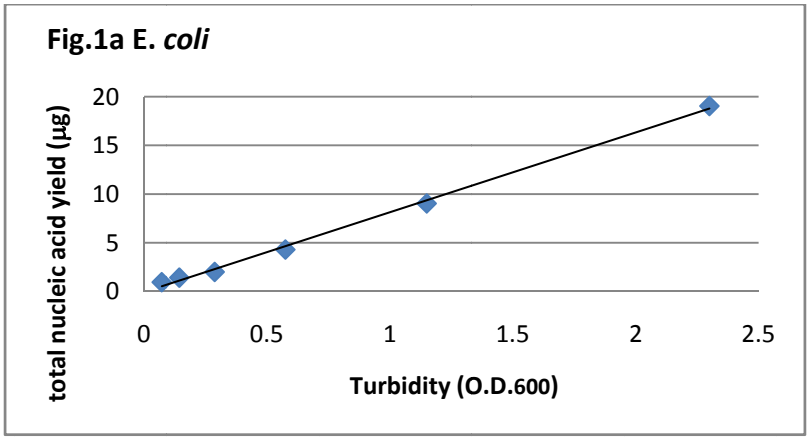
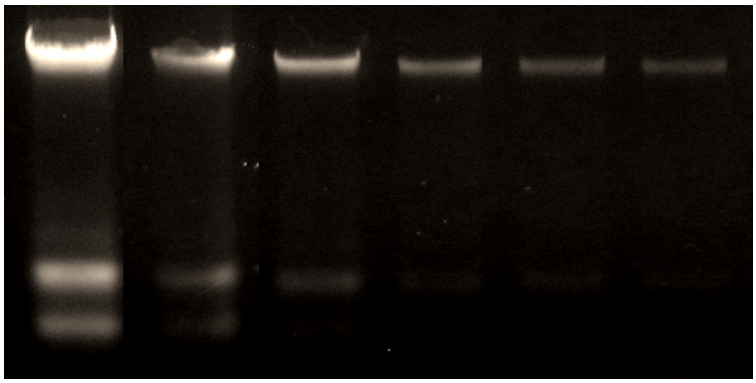


Fig.1b



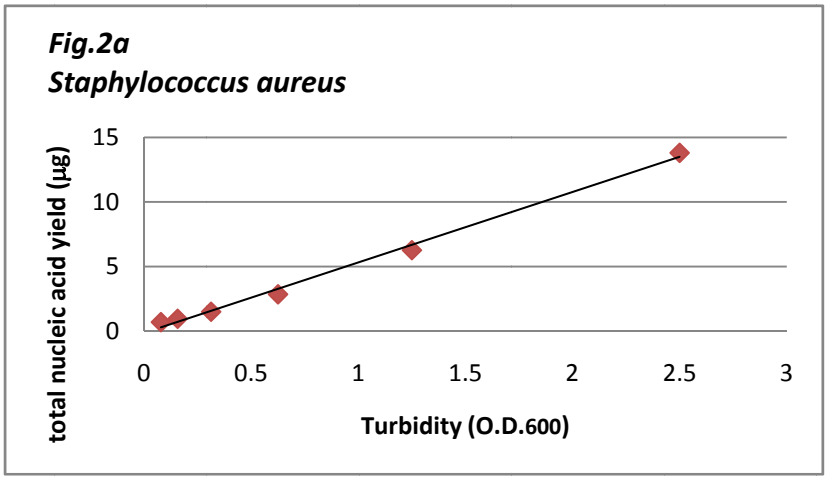
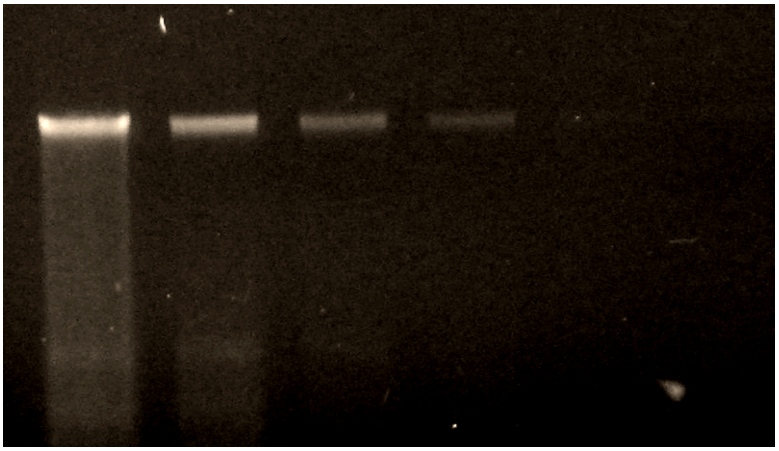


Fig.2b



(2) Sensitivity

Serial-dilution was performed on *Staphylococcus aureus* (ATCC27154) in the range of 10^9-10^1copy/ml). 200µl samples were extracted and eluted in 100µl. 25µl eluate was used for SYBR Green real-time PCR reaction which detected a *Staphylococcus aureus* specific gene. As few as 20 copies of bacteria (about 10^2 copy/ml in the sample, about 5 copy in PCR reaction) spiked can be detected, indicating excellent sensitivity and linearity of the isolation procedure (fig.3a and 3b)

Fig. 3a

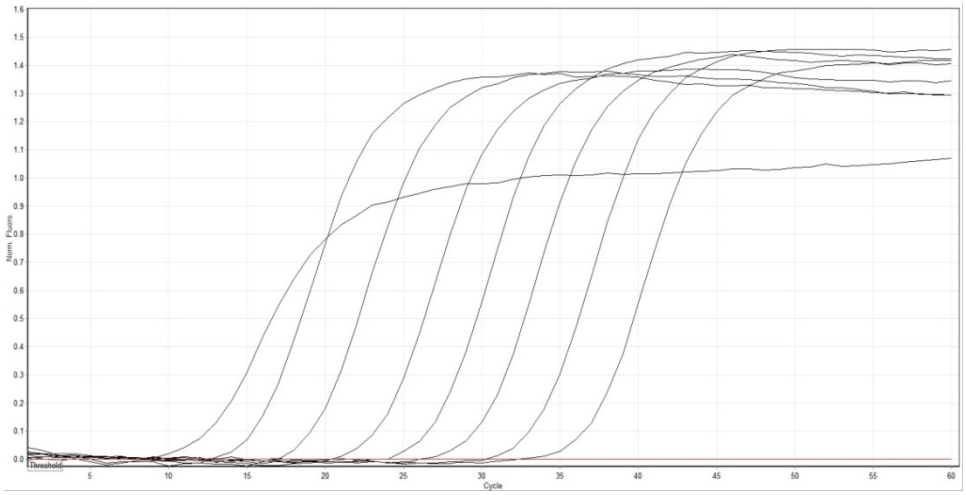
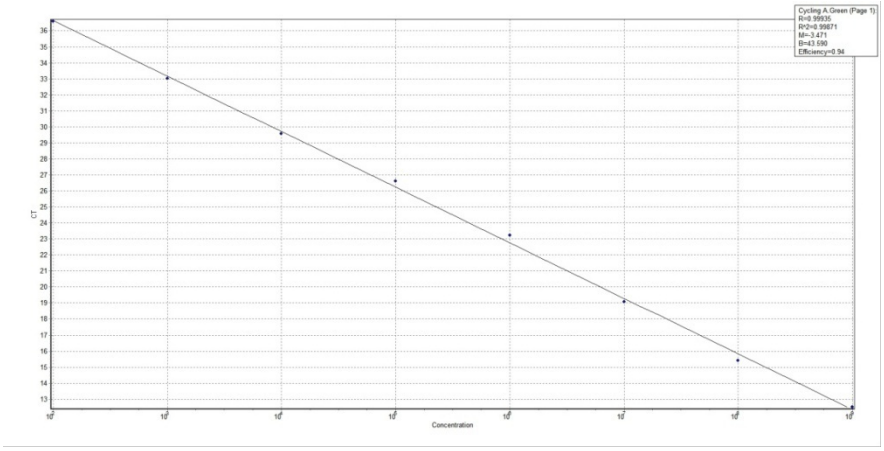


Fig.3b



Quality Control

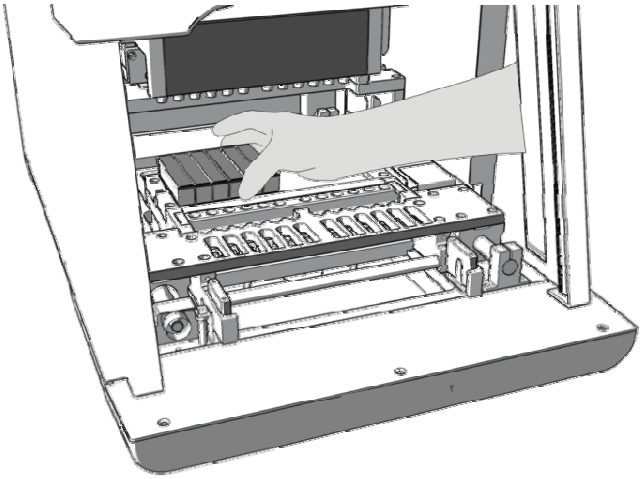
In accordance with BioChain's ISO-certified Quality Management System, each lot of AnaPrep Bacterial DNA Extraction Kits is tested to ensure consistency in product quality.

Protocol of extraction

1. Turn the power switch on and wait for the LCD screen to light up and display “AnaPrep 12 System Stand-By”.
2. Press the “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 the self-testing process.

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

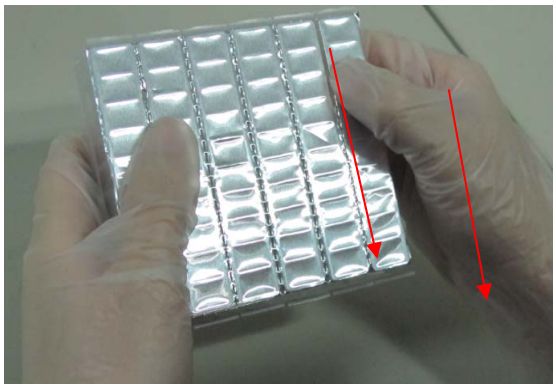
4. Load Reagent Cartridges, and all plastic disposables (Reaction Chamber, Tip Holder, Piercing Pin, and Filtered Tip)

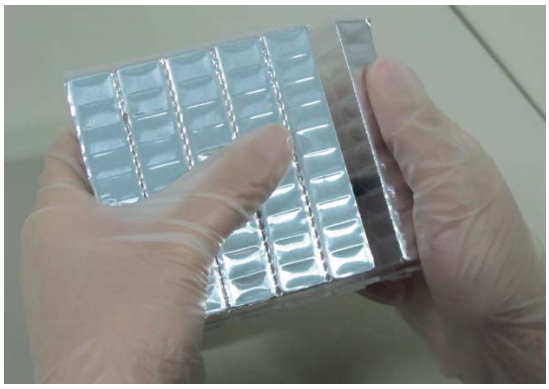


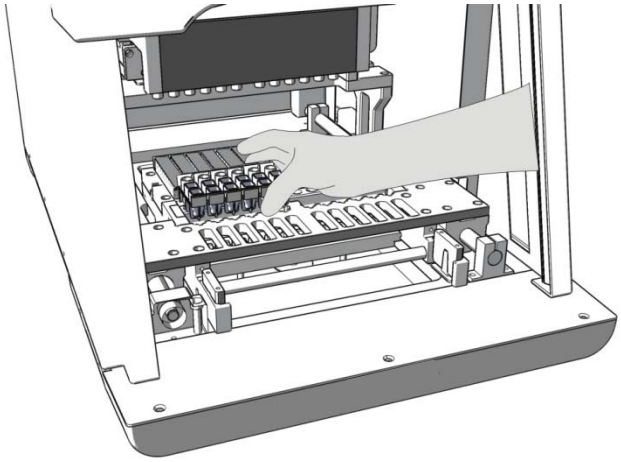
Insert Reagent Cartridges

■ How to pull apart the reagent cartridges

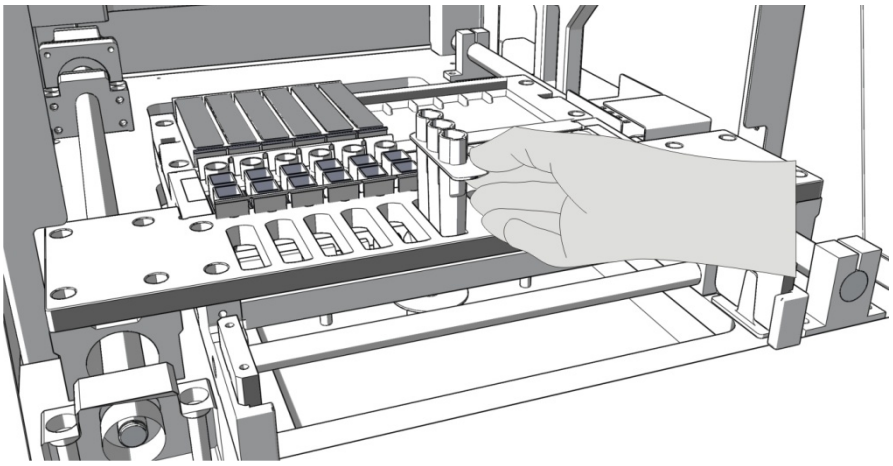
- Cut foil with a finger nail along the dotted line and then snap it apart with a little bit of force.



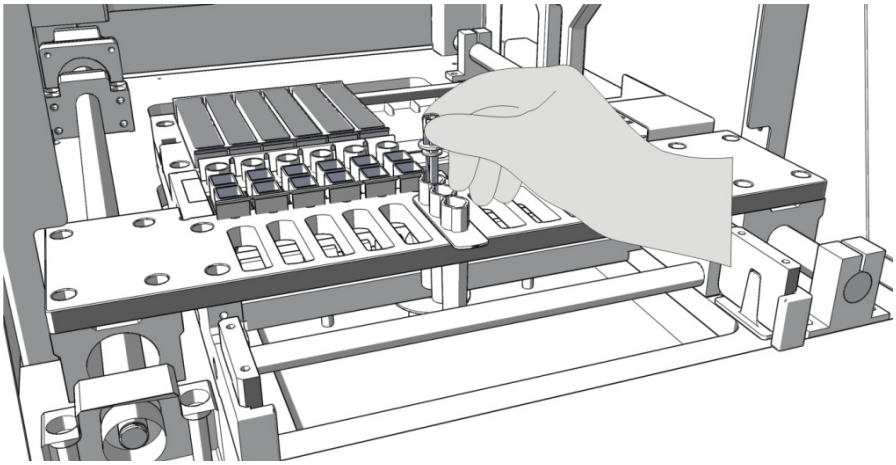




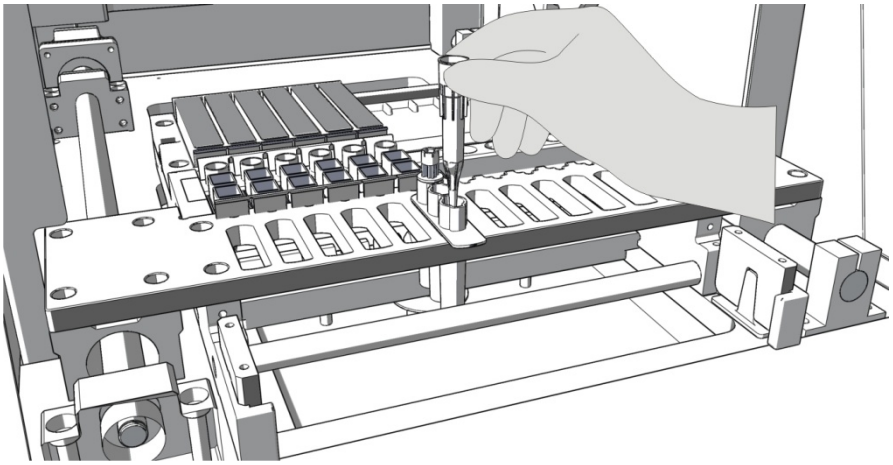
Insert Reaction Chambers



Insert Tip Holder



Insert Piercing Pins



Insert Filtered Tips

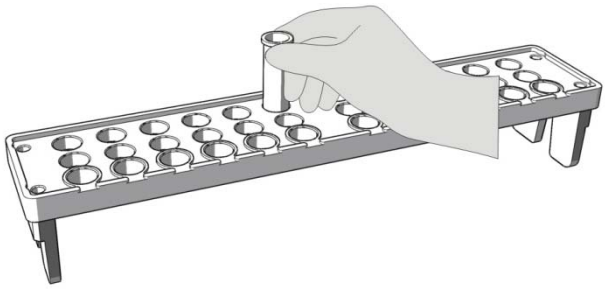
Note:

Load one Reagent Cartridge and one set of plastic disposables per sample.

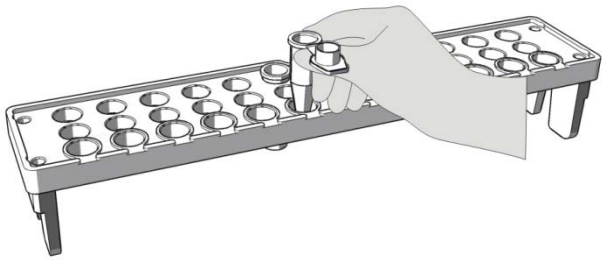
Important:

- Set Reagent 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.

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

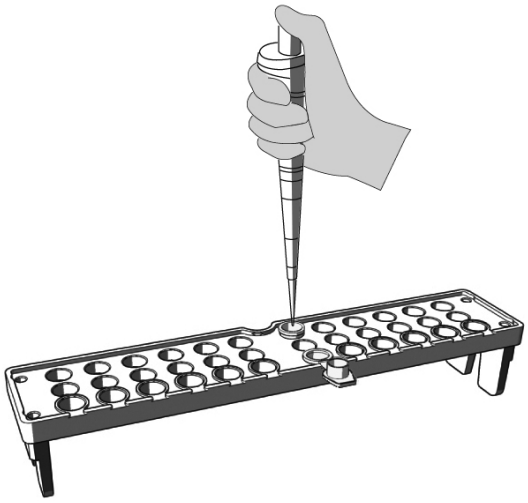


Insert Sample Tube into the Sample Rack



Insert Elute Tube into the Sample Rack

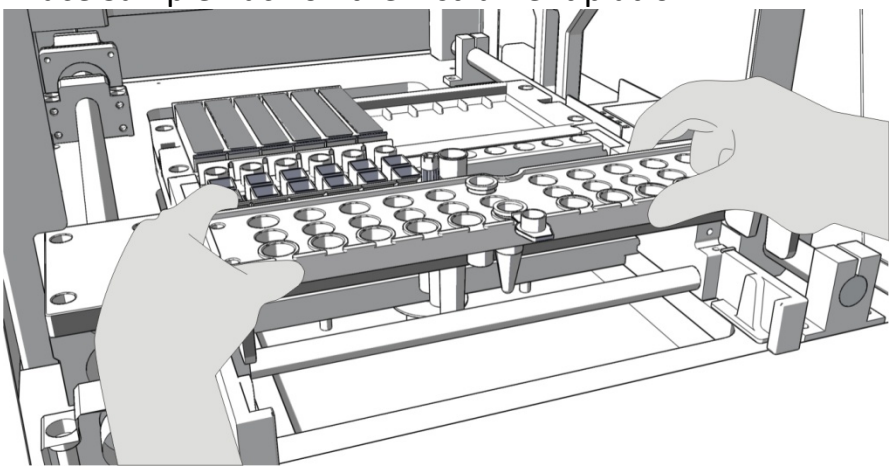
6. Load the sample(s) to Sample Tube.



Note:

- Pretreatments 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.

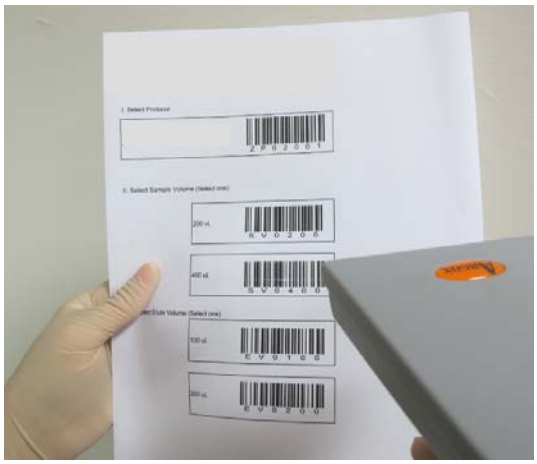
7. Place Sample Rack on the instrument platform



Note:

- Use two hands to handle the Sample Tray.
- Make sure the Sample Tray is placed correctly in the instrument.

8. Close the door.
9. Scan the protocol barcodes to select purification protocol, sample volume and elute volume.



Note:

- There is one protocol barcode paper enclosed in each reagent kit box.
- The protocol's name, sample volume and elution volume will be shown on LCD screen after the protocol barcodes are scanned.

10. Follow the instructions displayed on the LCD screen to double check the operation steps to be completed prior to running the program.
11. Press "Enter" to confirm. The instrument will start running the protocol program automatically and will terminate once all processes are completed.

    Note:

    - It takes 30 to 45 minutes to complete the extraction process and may vary according to reagent types.

12. At the end of the run, the instrument beeps briefly while the LCD screen displays "Protocol Completed".
13. Open the instrument door.
14. Remove the elute tubes containing the purified nucleic acids.
    Note: Store the purified nucleic acids at 4°C for short-term storage or store at -70°C for long-term storage.
15. Discard the used cartridges and all plastic consumables into the

biohazard waste. Do not reuse the cartridges

16. If you're not using the instrument, place the Sample Rack back into the AnaPrep, close the instrument door and press the "Start" button for 2 seconds to enter into "sleep mode".
    If the instrument will not be used for a longer period of time turn the power switch off.

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