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

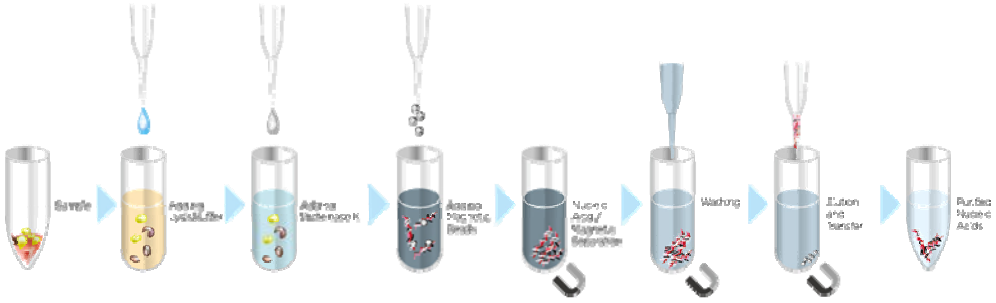
Introduction

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 on the AnaPrep 12 and 24 instruments for the preparation of nucleic acids from biological specimens. The AnaPrep instruments 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 instruments and reagent kits are suitable for a variety of polymerase chain reaction (PCR) tests. The AnaPrep instruments 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 products 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 BioChain's Technical Service Department or your local distributor.
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 Blood DNA Extraction Kit 200

Cat. No. Z1322001 Process Time: 50 minutes

Intended Use

The AnaPrep Blood DNA Extraction Kit is for use with the AnaPrep 12 or 24 instruments for extraction of DNA from 100-400 µl mammalian whole blood and buffy coat.

Application

Nucleic acids extracted with the AnaPrep Blood 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	Z1322001-48
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
Protocol Barcodes	1 pc

Reagent Cartridge Content

well-1	Proteinase K solution	40 µl
well-2	Lysis Buffer 2	1000 µl
well-3	Binding Buffer 1	600 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 Blood 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 total nucleic acid at 4 °C (short-term) or aliquot and store at –70°C (long-term).

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 2x 10^4 cells/µl)** ; 100-400 µl leukocyte concentration **(contains no more than 5x 10^6 cells)** ; 100-400 µl buffy coat** **NOTE**: *For samples containing a low leukocyte count (less than 1x10^3 cells/µl), concentrate the blood cells by centrifuging at 3000 r.p.m. for 15 min under 4°C. Then use the leukocyte concentration for DNA extraction. ** If the number of WBCs in the blood sample is more than 2 x10^4 cells/µl, use the AnaPrep blood DNA extraction kit 1200 or dilute the blood sample with PBS (e.g. the whole blood sample from lymphoma/myeloma patient or other granulocyte-rich blood sample/buffy coat).
Controls/Optional internal control#	Add controls /internal control in the extraction procedure if downstream analysis is needed (# see Controls/internal control on page 15)
Elute volume	50-300 µl

- If the sample volume is less, add the appropriate volume of PBS.
- The AnaPrep Blood DNA Extraction Kit has been proven to work with fresh or frozen blood samples collected in tubes containing common anti-coagulants like EDTA, heparin* and citrate. (*The EDTA is recommended to use as an anticoagulation agent, while heparin has inhibiting effects on nucleic acid amplification reactions).
- Using fresh whole blood samples (within 1 week, stored at 4-8°C) for extraction is recommended. The total nucleic acid yield and quality decreases over time. For long term storage, whole blood should be frozen and/or

aliqouted to prevent repeated freeze-thaw cycles.

- This protocol was established for isolating DNA from the whole blood of healthy individuals. Unhealthy or drug-treated individuals (ex. Patients of leukemia or infectious diseases) may show abnormal blood quality that may influence the nucleic acid extraction procedure.
- If using concentrated buffy coat (purified and free of blood), the AnaPrep Culture Cell DNA extraction kit (Z1322005) is recommended.
- If the whole blood sample is granulocyte-rich (white blood cell number more than 2 X 10^5 cells/μl), dilute the blood sample or extract the DNA by using the AnaPrep blood DNA extraction kit 1200 (Z1322002).
- 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, store purified DNA at 2–8°C for up to 3 days. Low levels of DNA degradation will occur after 3 days.
- For long-term storage, store the tubes at –70°C.
- The extracted product will contain total nucleic acid (DNA and RNA), but RNA is not the major product derived from this kit (about 10%) and will be degraded soon. If RNA-free product is needed, add some RNase to the eluate.

Result

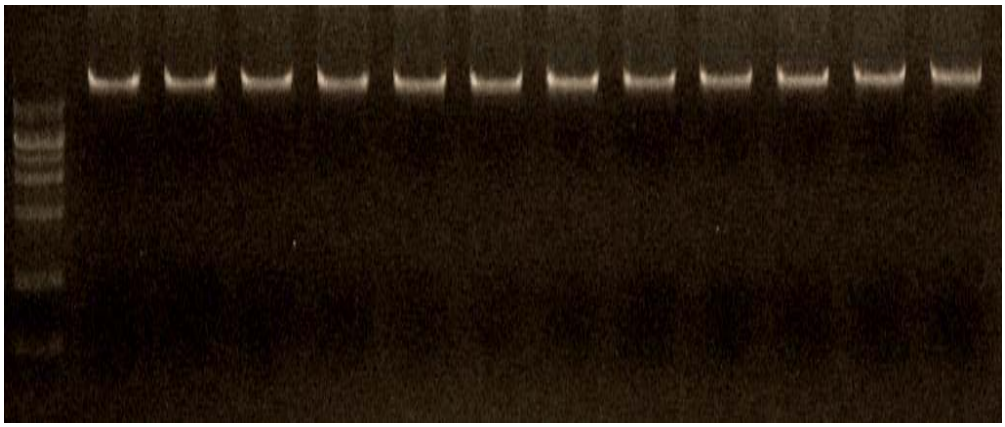
(1) Expected Purity and Yield

DNA was purified by using the AnaPrep Blood DNA extraction Kit 200 using five different human whole blood samples (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 AnaPrep Blood Extraction Kit 200 (from the blood sample of WBCs counts: 2-20x10^3 cells/μl). The DNA yield from whole blood depended on the specific blood donor and blood cell count. The yield from cultured cells was dependent on the type of cell line (due to the variable degree of aneuploidy).

Sample material	Volume/amount	DNA Yield	Purity
Whole Blood (WBC no. is 1.8 x 10^3 /ml)	100 μl 200 μl 300 μl 400 μl	1-1.2 μg 2-2.1 μg 2.8 -3.1 μg 4 -4.3 μg	OD260/OD280 >=1.7 OD260/OD230 >=1.5
Whole Blood (WBC no. is 4 x 10^3 /μl)	100 μl 200 μl 300 μl 400 μl	1.6 - 2.1 μg 3.8 - 3.9 μg 5.1 - 5.2 μg 8.5- 8.8 μg	
Whole Blood (WBC no. is 6.9 x 10^3 /μl)	100 μl 200 μl 300 μl 400 μl	2.9 - 3.1 μg 5.8- 6.2 μg 8.2 -8.8 μg 11.9 - 12.5 μg	
Whole Blood (WBC no. is 10.9 x 10^3 /μl)	100 μl 200 μl 300 μl 400 μl	4.3- 4.6 μg 8.7-9.1 μg 11.5-12.2 μg 16.6- 17.5 μg	
K562 cells	6 x 10^5 cells 2 x 10^6 cells	9-9.6 μg 22-25 μg	

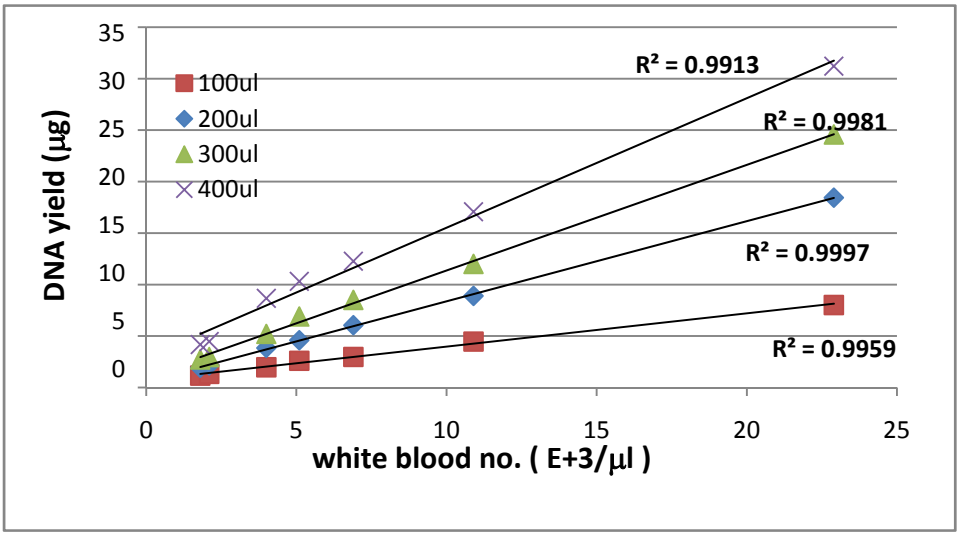
(2) Integrity

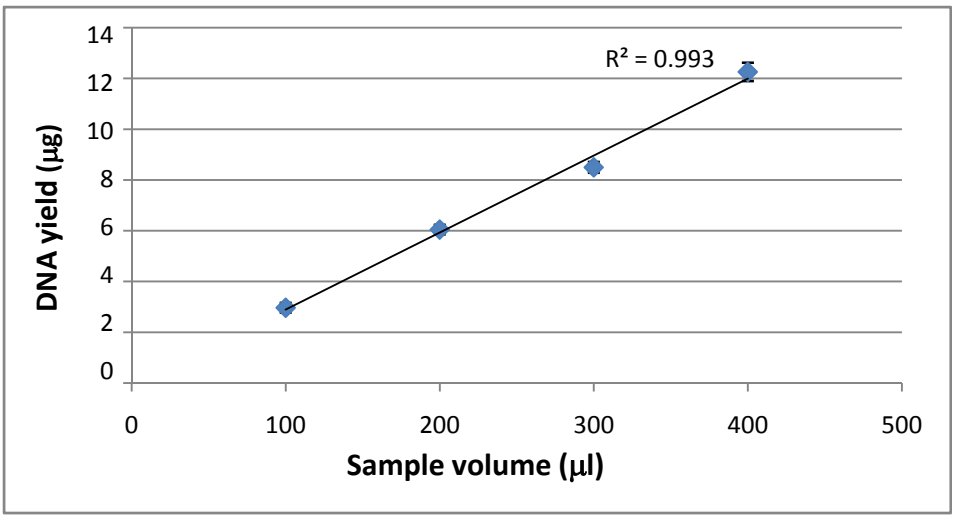
DNA was isolated in replicates of 12 from 200 µl of human whole blood taken from two different donors. Elution volume was set to 100 µl. Integrity of DNA was shown by subjecting each eluate to TAE agarose gel electrophoresis together with a lambda/hind Ⅲ marker (of fragments with size: **0.56, 2.02, 2.32, 4.36, 6.56, 9.42, 23.13**kb). All samples exhibited a single band with a molecular weight more than 22kb and without smears.



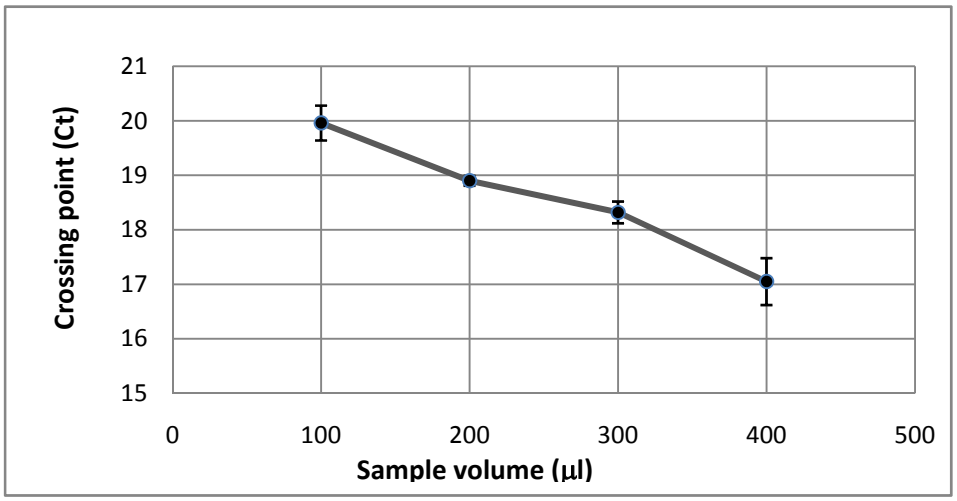
(3) Scalability

A. DNA was isolated from different whole blood samples (WBCs count range 1.8 – 22 X 10^3 cells/µl). The DNA yield (measured by Nanodrop 2000 UV-Vis spectrophotometer) shows excellent scalability with different sample volumes (100, 200, 300 and 400 µl) and WBCs count.



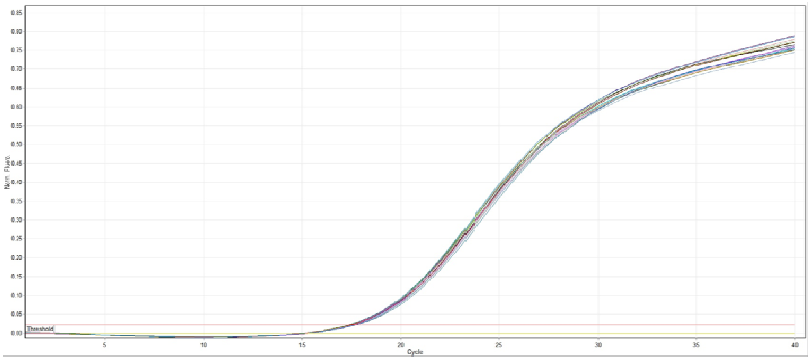


B. DNA isolated from different amounts of whole blood samples were amplified by real-time qPCR using β-globin gene specific primers. The resulting crossing points confirm the scalability on extraction.



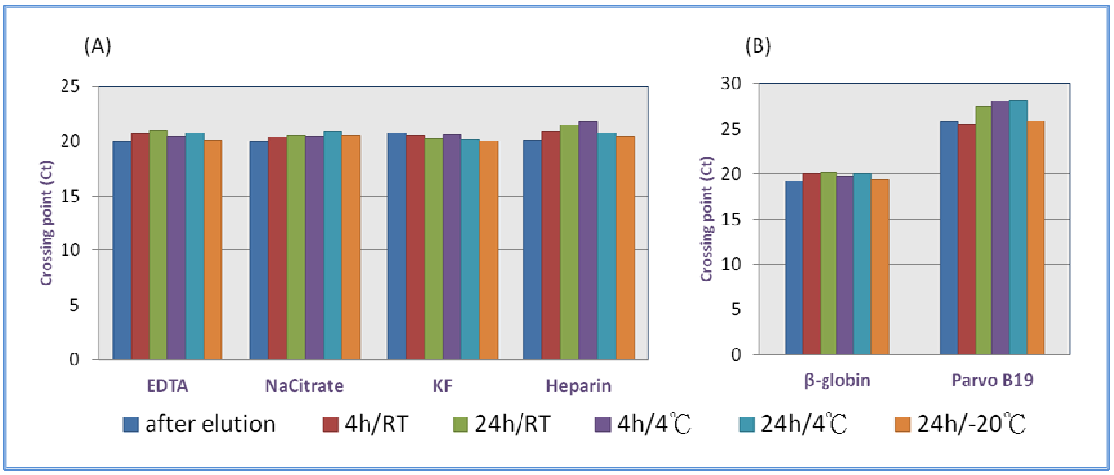
(4) Reproducibility

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



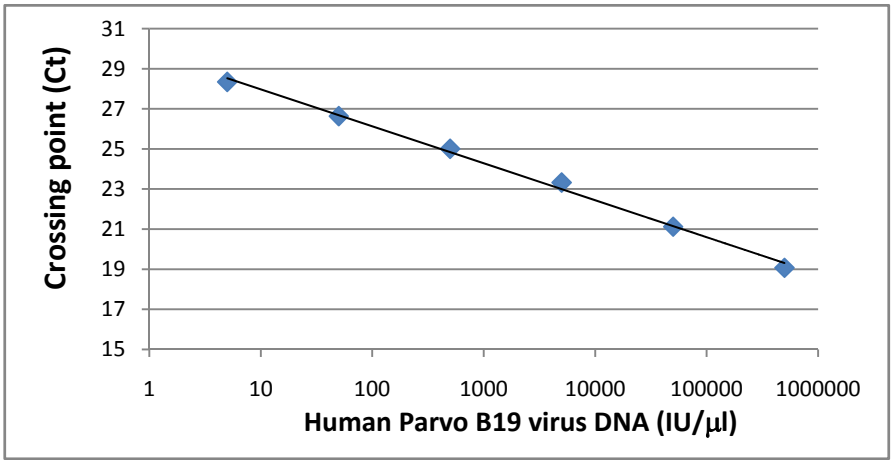
(5) Stability

DNA was extracted from whole blood using the AnaPrep Blood DNA Extraction kit 200. Real-time qPCR detection of β-globin (A) and spiked parvovirus B-19 DNA (A,B). No significant differences were observed between samples with different anticoagulants and storage conditions.



(6) Sensitivity

Results showing whole blood (in EDTA collection tubes) spiked with serial-diluted human Parvo B19 Virus (in range of 5-500000 IU/µl). 200 µl of the samples were extracted and eluted in 100 µl. 10 µl of the eluate was then used for real-time PCR reaction by RealStar® Parvovirus B19 PCR kit 1.0. As little as 5 IU spiked B19 virus (about 1 IU in PCR reaction) in the sample can be detected, indicating the excellent sensitivity and linearity of the isolation procedure.



Controls/ internal control

Using appropriate controls for downstream analysis: :

Type	Description	Location
Positive control	**Using a sample that is positive for the target**	**Place in sample tube**
Negative control	**Using a sample that is negative for the 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**

Quality Control

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

Protocol of extraction

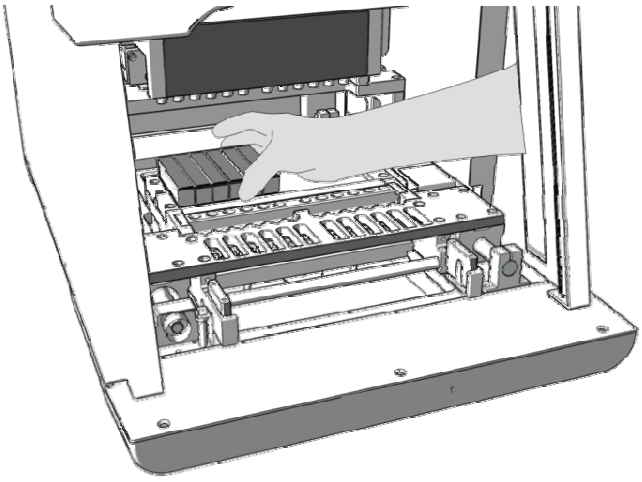
1. Turn the power switch on and wait for the LCD screen to light up and display “AnaPrep 12 System Stand-By” or “AnaPrep 24 System Stand-By” (figures shown here are from AnaPrep 12 and both systems operate the same way).
2. Press the “Start” button
   (The system will process self-testing, and then go 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.

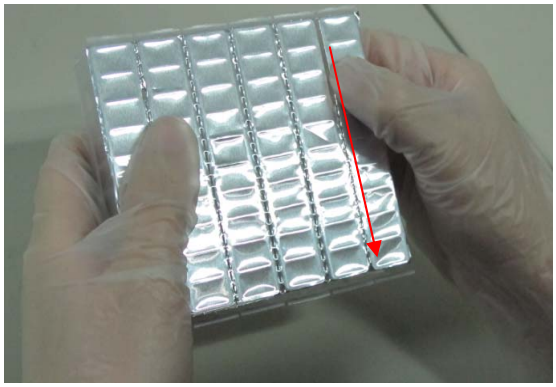
4. Load Reagent Cartridges, and all plastic disposables (Reaction Chamber, Tip Holder, Piercing Pin, and Filtered Tip).

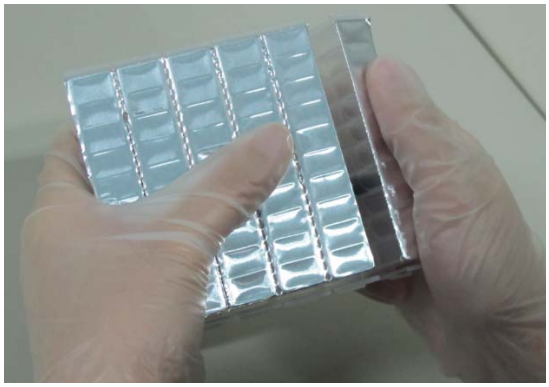


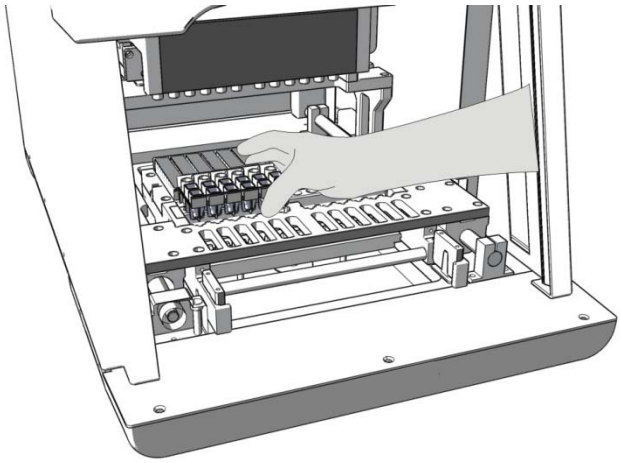
Insert Reagent Cartridges

■ How to pull apart the reagent cartridges

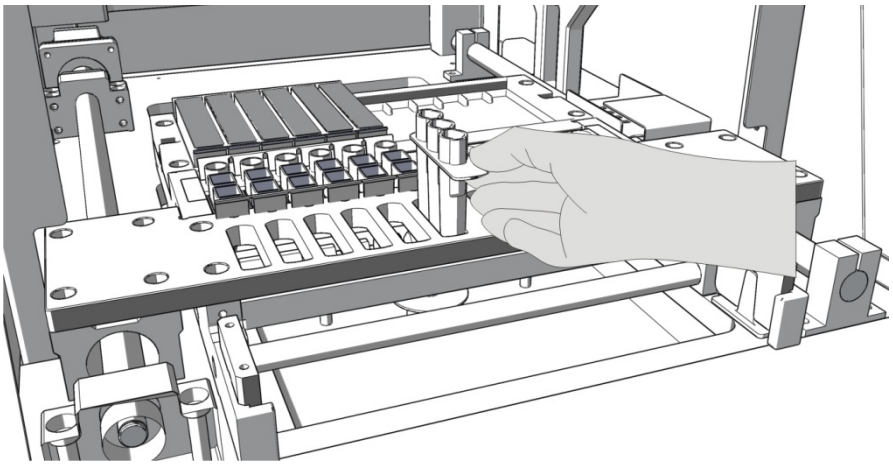
- Cut foil with a finger nail along the dotted line and then snap it apart with a little bit of force.



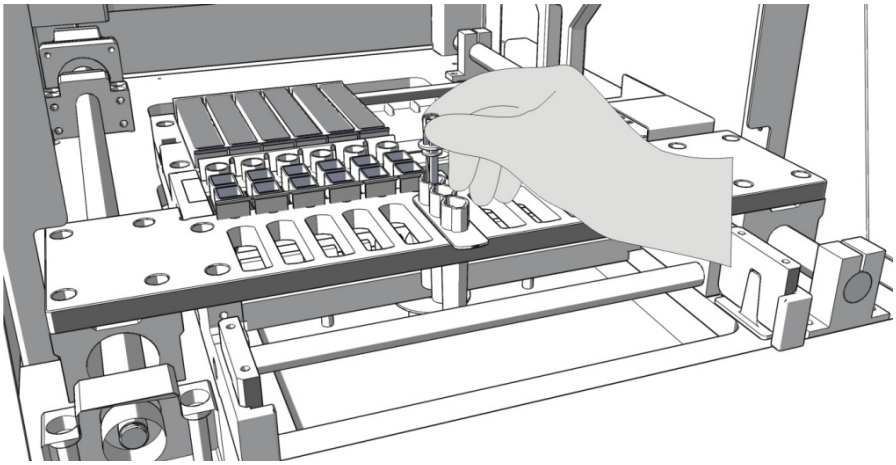




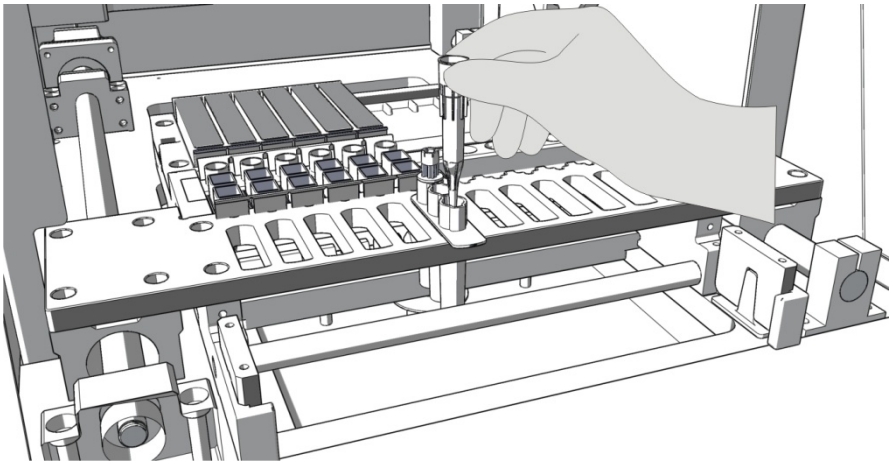
Insert Reaction Chambers



Insert Tip Holder



Insert Piercing Pins



Insert Filtered Tips

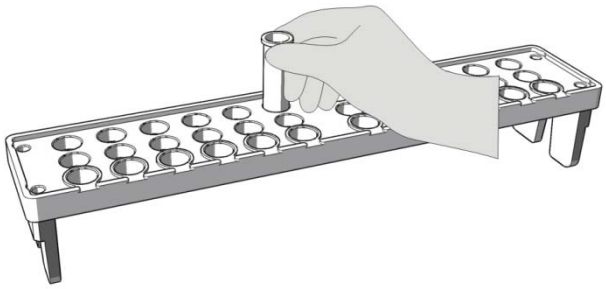
Note:

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

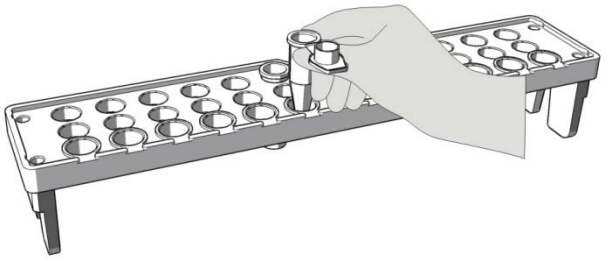
Important:

- 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 (1-24 for AnaPrep 24) 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

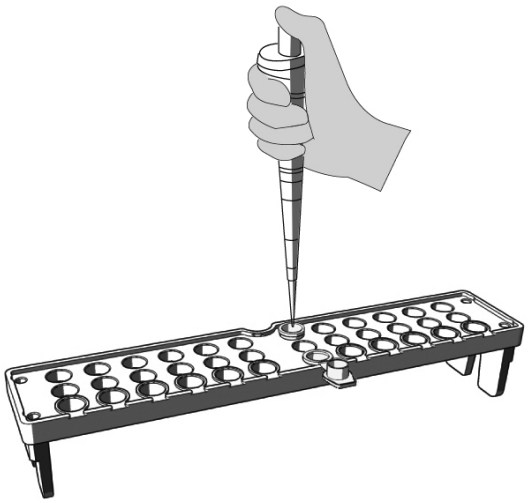


Insert Sample Tube into the Sample Rack



Insert Elute Tube into the Sample Rack

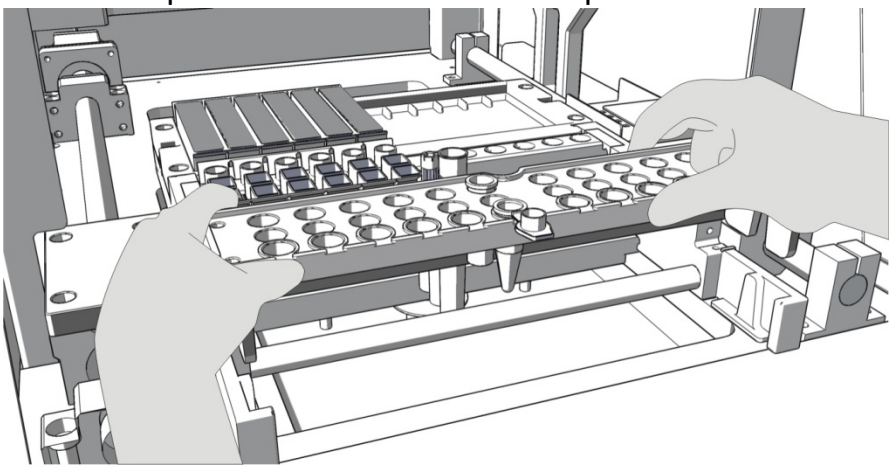
6. Transfer the sample(s) to Sample Tube.



Note:

- Pretreatments are essential for some sample types before transferring to Sample Tube. Please refer to the handbook of reagent kits for details.
- Make sure the caps of Elute Tube are open as shown in the figure above.

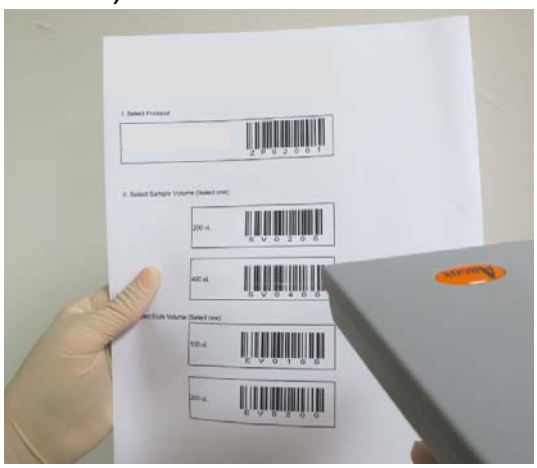
7. Place Sample Rack on the instrument platform



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 75 minutes to complete the extraction process and varies according to reagent types.

12. At the end of the run, the instrument will beep 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-off the power switch.

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