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
RNA	Total RNA	Cell culture, human and animal tissue (fresh and frozen tissues), fresh whole blood	See Reagent Handbook	AnaPrep Total RNA Extraction Kit with DNase-Treatment

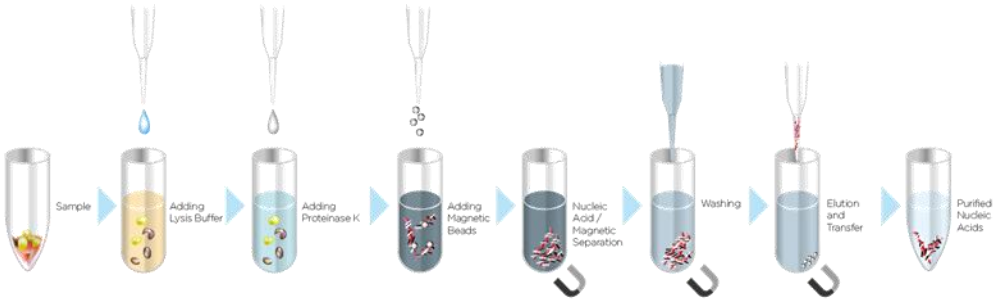
Introduction

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 Total RNA Extraction Kit with DNase-Treatment

Cat. No. Z1322017 Process Time: 75 minutes

Intended Use

The AnaPrep Total RNA Extraction Kit with DNase-Treatment is designed for use with the AnaPrep 12 instrument for extraction of total RNA from cell lines, tissues, and fresh blood samples.

Application

Nucleic acids extracted from the Total RNA Extraction kit can be used in a number of downstream applications including: RT-PCR, cDNA library construction, and Northern Blot Analysis.

Number Of Tests

48 extractions

Kit Components

Kit Contents	Z1322017
Reagent Cartridge	48 pcs (4x6x2)
Reaction Chamber	48 pcs (4x6x2)
Tip Holder	48 pcs (24x2)
Filtered Tip	50 pcs (50x1)
Piercing Pin	50 pcs (50x1)
Filter Column	50 pcs (50x1)
Collection Tube	50 pcs (50x1)
Elute Tube (1.5 mL)	150 pcs (50x3)
Buffer RFC	1 pc (50mL x1)
RNA H	1 pc (10mL x1)

Reagent Cartridge Content

well-1	Empty	
well-2	Lysis Buffer 2	720 µl
well-3	Binding Buffer 1	1000 µl
well-4	Magnetic Bead Solution	800 µl
well-5	Washing Buffer 1	1200 µl
well-6	Washing Buffer 2	1000 µl
well-7	Washing Buffer 3	1000 µl
well-8	RNase-free water	1000 µl
well-9	RNase-free water	1000 µl
well-10	Empty	

Storage

- The AnaPrep Total RNA Extraction Kit should be stored at room temperature (15-25°C), except for the RNA H solution which should be stored at 2-8°C upon receipt. Do not freeze the reagent cartridges. The Kits are stable for 12 months under the proper storage conditions.
- Store the purified RNA at -70°C.

Starting Material

- **Whole blood**
  1. For optimal results, blood samples should be kept at 4°C and processed within a few hours.
  2. Perform erythrocyte (RBC) lysis procedure before extraction.

- **Tissues and Culture Cells**
  1. To prevent degradation by intracellular RNase, flash-freeze tissues and store at -70°C, or process immediately upon excision.
  2. Use an RNA-stabilizing reagent (e.g. RNA later) if the sample cannot be frozen immediately.
  3. Cells may be collected as pellets and flash-frozen in liquid nitrogen and stored at -70°C, or processed immediately.

- The amount and type of starting material required for use in the AnaPrep Total RNA purification procedure is shown in Table listed below.

Sample Type	Target Nucleic Acid	Sample volume (Amount of starting material)	Elution Volume
Whole Blood	Total RNA	200-400 µl /About 10^6 WBCs	50-200 µl
Tissue		10-100 mg tissue	
Cultured cells		Up to 10^7 cells	
NOTE: RBC lysis should be performed if using whole blood samples			

Sample Preparation

- Before beginning protocol, add 10 µl of β-mercaptoethanol (β-ME) to every 1 ml of RFC buffer and chill on ice until ice-cold.
- The table below describes the sample preparation procedures

---

Table:
Sample Preparation Procedures

Sample type	Procedure
Whole blood* * if using Paxgene blood RNA tubes, process according to manufacturer's instructions until the blood cell pellet is obtained and then proceed directly to step 6 of this protocol	1. Freshly prepare 1x RBC lysis buffer <u>10x RBC lysis buffer stock solution</u> 1.5 M NH4Cl 100 mM $KHCO_3$ 10 mM Na_2EDTA Adjust to pH 7.2 – 7.4 using HCl Filter at 0.2 mm, and store at 4°C for up to 6 months 2. Add two volumes of ice-cold 1x RBC lysis buffer to one volume of blood sample 3. Invert 3-5 times and incubate on ice for 10-15 min 4. Centrifuge at 1000 x g for 10 min at 4°C 5. Remove supernatant 6. Add 1 ml RFC Buffer to pellet and homogenize (use Elution tube included in kit) 7. If using difficult to fully homogenize sample,

	pulse to 5000 rpm after homogenizing and transfer supernatant to new tube to remove non-homogenized sample (use Elution tube included in kit) 8. Incubate at -20^{o}C overnight 9. Proceed to “Loading AnaPrep” protocol
Tissues and Cultured Cells	1. Take 10-40 mg tissue or cell pellet and homogenize in 1 ml RFC buffer (use Elution tube included in kit) 2. If using difficult to fully homogenize sample, pulse to 5000 rpm after homogenizing and transfer supernatant to new tube to remove non-homogenized sample (use Elution tube included in kit) 3. Incubate at -20^{o}C overnight 4. Proceed to “Loading AnaPrep” protocol
Loading AnaPrep	1. Spin overnight samples at 13000 rpm and 4^{o}C for at least 5 min. Carefully remove supernatant as much as possible without disturbing the pellet 2. Add 200 ul RNA H buffer to pellet 3. Cut off lid of tube and place on Anaprep sample rack in SAMPLE ROW 4. Use another Elution tube provided in kit and add the following: 53 µl RNAse-free water + 7 µl 10x DNase buffer + 10 µl DNase (to be user-supplied). Cut off lid of tube and place on Anaprep sample rack in MIDDLE ROW 5. Place empty elution tube (no need to cut off lid)

	on Anaprep sample rack in ELUTION ROW
Programming AnaPrep	1. After turning on AnaPrep, press Start to initialize. "Select Protocol, read barcode" will then show up on the screen. The protocol must be entered manually 2. Press "0" and the instrument will ask for a 4-digit code. Type "8828" and press "Enter" 3. The program will ask for a sample volume. Press "0" and then enter the volume in microliters. Press "Enter" 4. The program will ask for an elution volume. Press "0" and then enter the desired volume in microliters. 5. The program will ask for the DNase incubation time in seconds. Input up to 999 depending on the time of incubation desired. Press "Enter" 6. The program will ask for the heating temperature. If 37^{o}C DNase incubation is desired, input "45." (The actual reaction chamber is adjacent to the heating well, so a 45^{o}C heat setting will result in a 37^{o}C incubation). If room temp DNase incubation is desired, input "33." (Note: the instrument will NOT run if a smaller number is entered) Press "Enter" 7. Press "Enter" again to start the extraction.

Yield of purified RNA	RNA yields depend on the sample type and amount used as input

Result

(1) Scalability

The AnaPrep Total RNA Extraction Kit with DNase-treatment was used to extract RNA from cultured Jurkat cells at different cell counts (Figure 1). The results indicate that the total RNA extraction has excellent scalability.

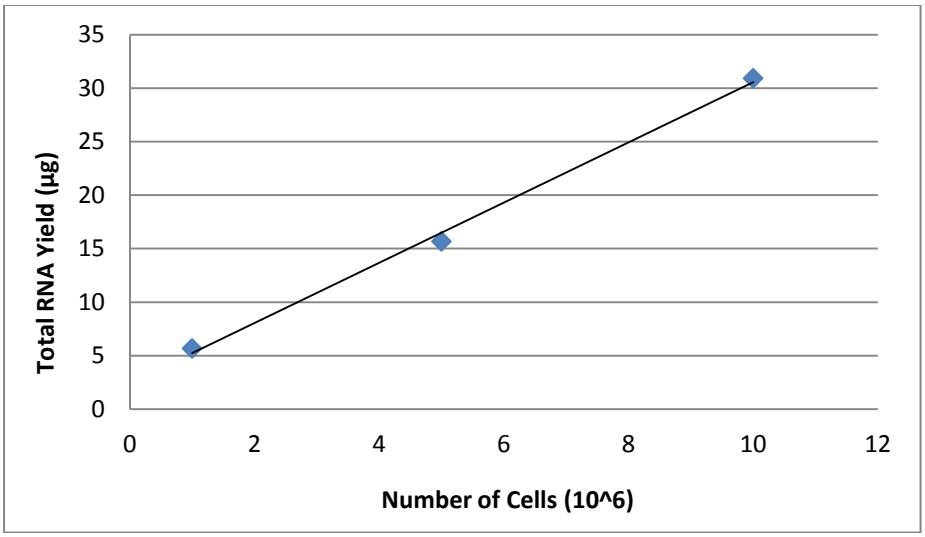


Figure 1. The AnaPrep Total RNA Extraction kit with DNase-treatment has excellent scalability. $1x10^7$, $5x10^7$, and $1x10^8$ Jurkat cells were collected, and the RNA was extracted with a final elution volume of 50 µl.

(2) Quality

High-quality RNA is obtained using our Total RNA Extraction Kit with DNase-treatment. The samples show strong intact 28S/18S bands when run on a denaturing gel (Figure 2), with A260/280 ratios consistently between 1.9 – 2.1 and A260/230 ratios ~2.0, indicating highly pure RNA.

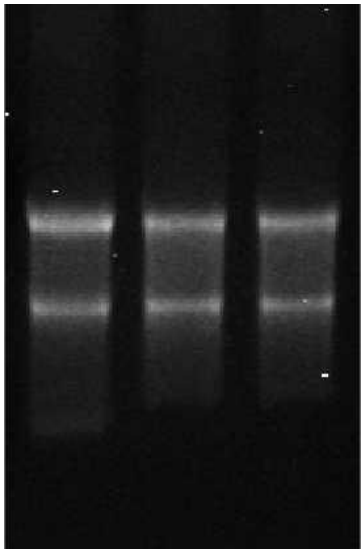


Figure 2. The AnaPrep Total RNA Extraction kit with DNase-treatment yields high-quality RNA samples. From left to right: (1) Jurkat RNA obtained using traditional phenol-chloroform methods, (2) and (3) Jurkat RNA obtained using our automated RNA Extraction kit

Quality Control

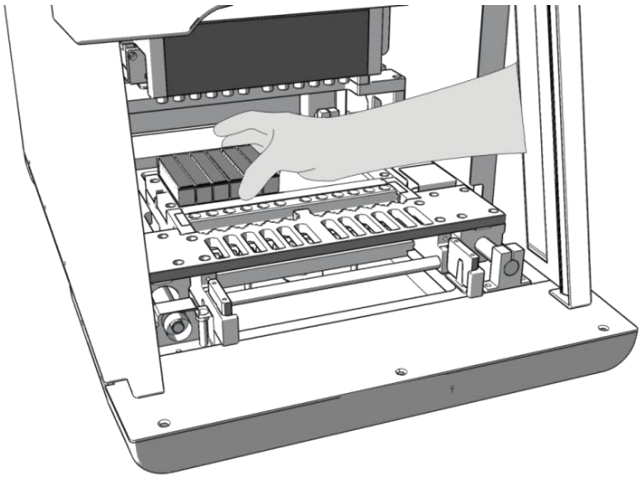
In accordance with BioChain's ISO-certified Quality Management System, each lot of AnaPrep Bacterial DNA Extraction Kits is tested to ensure consistency in product quality.

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.

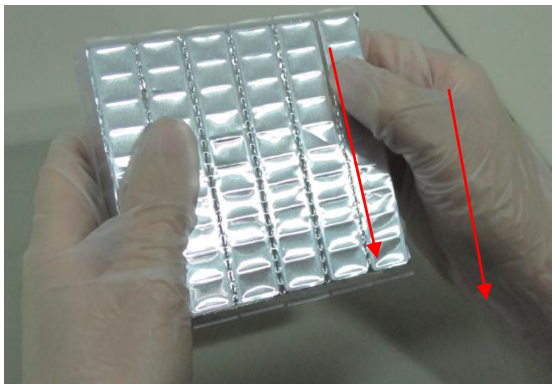
4. Load Reagent Cartridges, and all plastic disposables (Reaction Chamber, Tip Holder, Piercing Pin, and Filtered Tip)

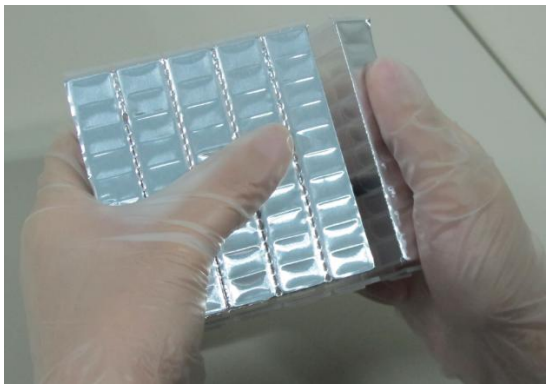


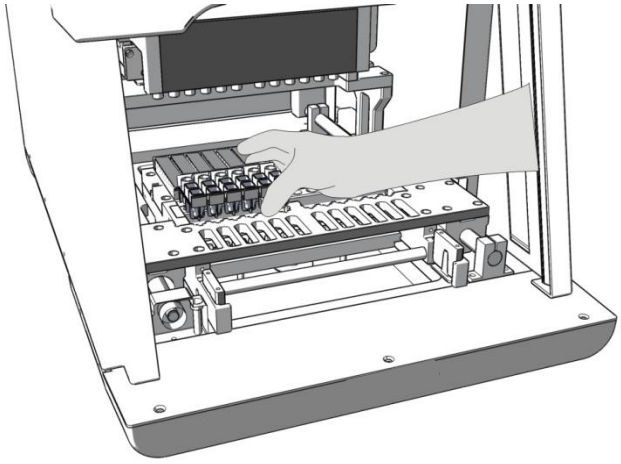
Insert Reagent Cartridges

■ How to pull apart the reagent cartridges

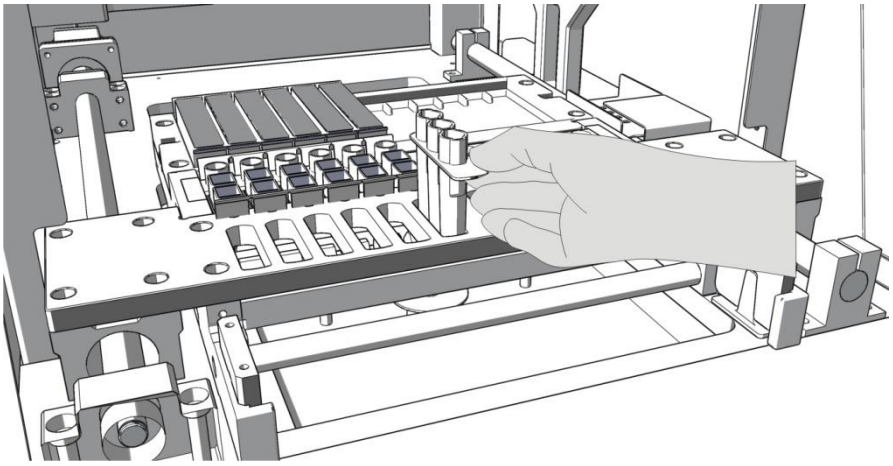
- Cut foil with a finger nail along the dotted line and then snap it apart with a little bit of force.



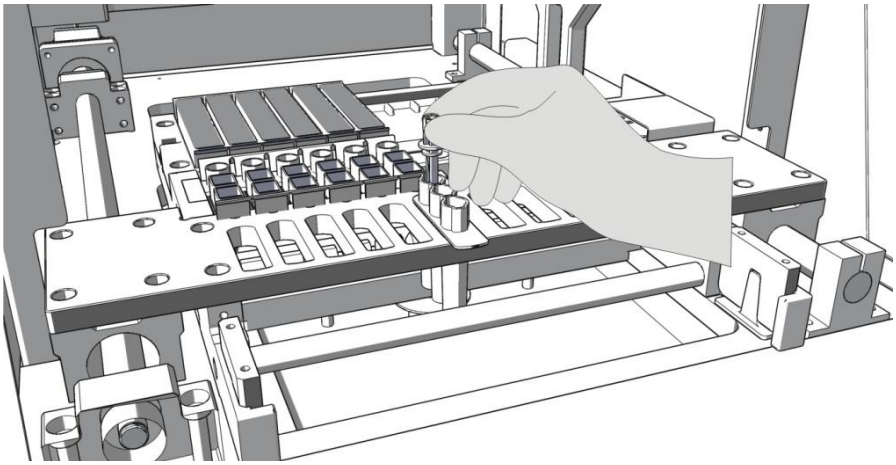




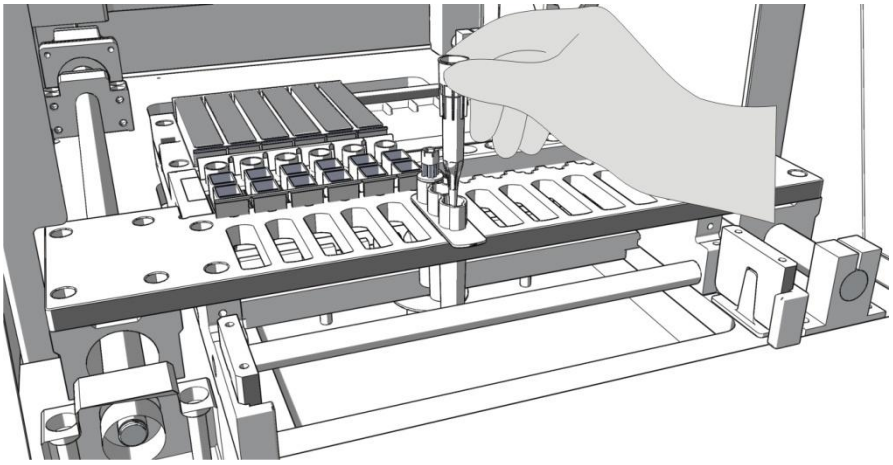
Insert Reaction Chambers



Insert Tip Holder



Insert Piercing Pins



Insert Filtered Tips

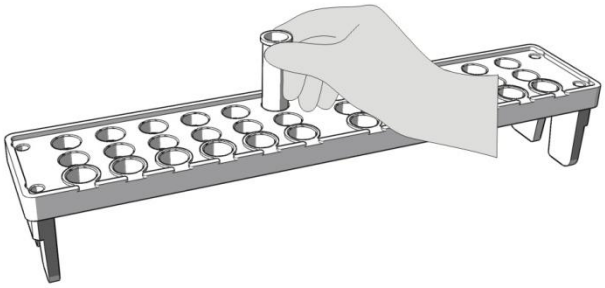
Note:

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

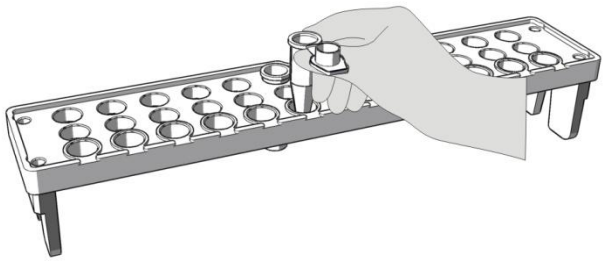
Important:

- 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

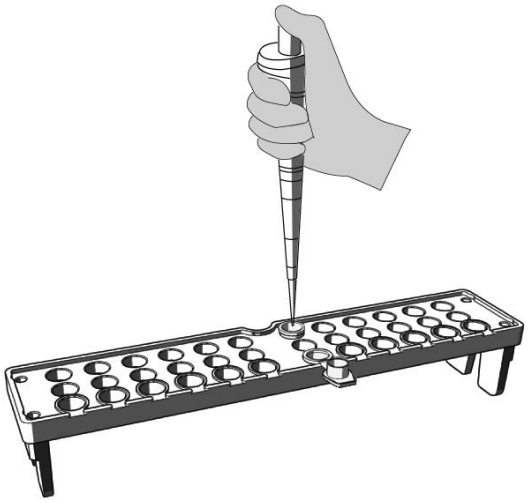


Insert Sample Tube into the Sample Rack



Insert Elute Tube into the Sample Rack

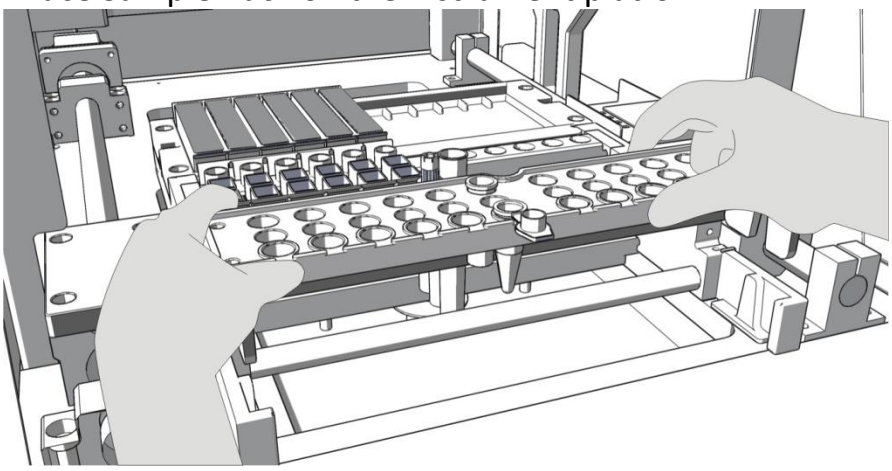
6. Load the sample(s) to Sample Tube.



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. Follow procedures in “Programming AnaPrep” section above.
10. The instrument will start running the protocol program automatically and will terminate once all processes are completed.
11. At the end of the run, the instrument beeps briefly while the LCD screen displays “Protocol Completed”.
12. Open the instrument door.
13. Remove the elute tubes containing the purified nucleic acids.
    Note: Store the purified nucleic acids at -70°C.
14. Discard the used cartridges and all plastic consumables into the biohazard waste. Do not reuse the cartridges.
15. 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.

BioChain Institute Inc.

www.BioChain.com
39600 Eureka Dr.
Newark, CA 94560, USA
Tel: 1-510-783-8588
Fax: 1-510-783-5386
Mail: info@BioChain.com