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

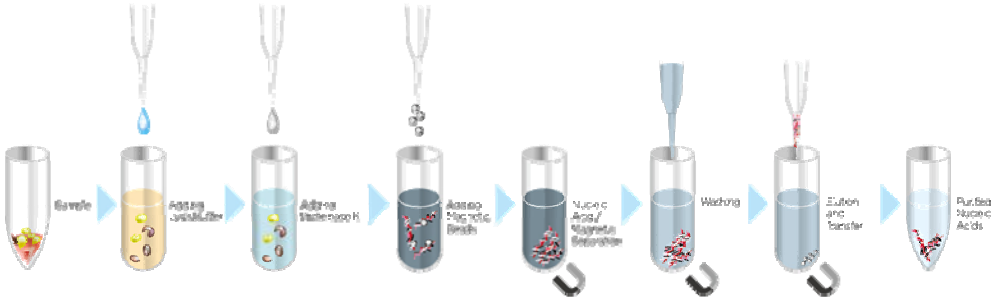
Introduction

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). The AnaPrep instruments and reagent kits are intended for laboratory 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 FFPE DNA Extraction Kit

Cat. No. Z1322009 Process Time: 45 minutes

Intended Use

AnaPrep FFPE DNA Extraction Kit is used with the AnaPrep 12 or 24 for extraction of genomic DNA from FFPE (Formalin-Fixed, Paraffin-Embedded) tissue samples. Providing good quality, high integrity DNA for Molecular diagnosis and research.

Application

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

Number Of Tests

48 extractions

Kit Components

Kit Contents	Z1322009-48
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
Filter Column	50 pcs
Collection Tube	50 pcs
Protocol Barcodes	1 pc
Dewaxil (28 ml)	1 pc
Proteinase K (20mg/mL) (1.1 ml)	1 pc
FFPE Lysis Buffer (10 ml)	1 pc

Reagent Cartridge Content

well-1	Empty	
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

- AnaPrep FFPE DNA Extraction Kit should be stored at room temperature (15-25 °C). Store Proteinase K at -20°C. Do not freeze the reagent cartridges. The Kits are stable for 12 months under the proper storage conditions.
- Store purified DNA at 4 °C (short- term) or aliquot and store at -70°C (long-term).

Starting Material

- FFPE (formalin fixed Paraffin Embedded) tissue samples: one to five section(s) with 6-10μm in thickness.

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

*Note: The surface area of tissue (not thickness) should be more than 1 x 1 cm_2, if the area is smaller, use more than one section for extraction.

Yield of purified DNA

- DNA yields depend on the sample type, number of nucleated cells in the sample, and the thickness of the section.
- Table listed below shows DNA yields obtained from different sample types using AnaPrep extraction procedures.

Table: The DNA yield of different sample types (tissue)

Sample Type	Sample Amount	Typical DNA Yield
Skeletal muscle	200 µl (40 mg tissue digested)	Up to 9µg
Heart	200 µl (20 mg tissue digested)	Up to 12µg
Spleen	200 µl (10 mg tissue digested)	Up to 27µg
Lung	200 µl (10 mg tissue digested)	Up to 17µg
Kidney	200 µl (10 mg tissue digested)	Up to 18µg
Liver	200 µl (10 mg tissue digested)	Up to 40µg
Buccal cells	1 Swab	1-5 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.

- DNA from FFPE tissue samples is often fragmented which causes problems in molecular assay. Keeping the integrity of the DNA is most important thing in the whole procedure.

- DNA fragments may cause high readings at OD260 resulting in inaccurate 260/280 ratios. The concentration should be estimated using DNA dyes rather than with a spectrophotometer. A better method for the integrity check is performing a PCR reaction of house-keeping genes with different length of products.

Sample preparation

1. Transfer sections

Transfer sections of paraffin-embedded tissue into 1.5 ml microcentrifuge tubes (not supplied). For most tissue types, curls/scrolls of one to four sections at 6-10 µm thickness is recommended; however, up to 10 very small sections such as needle biopsies may be used.

2. Add Dewaxil

Add 500ul De-paraffin reagent to the sample. Incubate at 90°C for 2 minutes. Vortex to mix.

3. Add Lysis Buffer

Add 200 µl FFPE Lysis Buffer into the sample tube and vortex to mix. Spin at 10,000 × g for 30 seconds at room temperature. Two phases will be formed, a lower (aqueous) phase and an upper (Dewaxil) phase.

4. Add Proteinase K

Add 20 µl Proteinase K directly to the lower phase and mix only the lower phase by pipetting up and down 20-30 times.

5. Incubate

Incubate the sample at 56°C for 1.5 hours, and then incubate the sample tube at 90°C for 1 hour in a water bath or thermomixer with periodic mixing.
Note: The first 1.5 hour is the critical step for proteinase lysis. Incubating the sample with periodic mixing (every 30 min) of the lower phase is necessary for complete digestion. Increasing the incubation time is not necessary for lysis because the proteinase activity drops after 2 hours. If the tissue sample is thick, adding more proteinase is recommended.

6. Centrifuge and filtration

Centrifuge the sample tube briefly to collect any drops from the inside of the lid and then carefully transfer the lower phase (about 220 µl) to the provided filter column that is sitting in the collection tube (avoiding white paraffin residues). Centrifuge at 6000 x g for 1 min.

7. Collection

Transfer 200 µl of the filtrate from the collection tube to a sample tube for extraction.

Quality Control

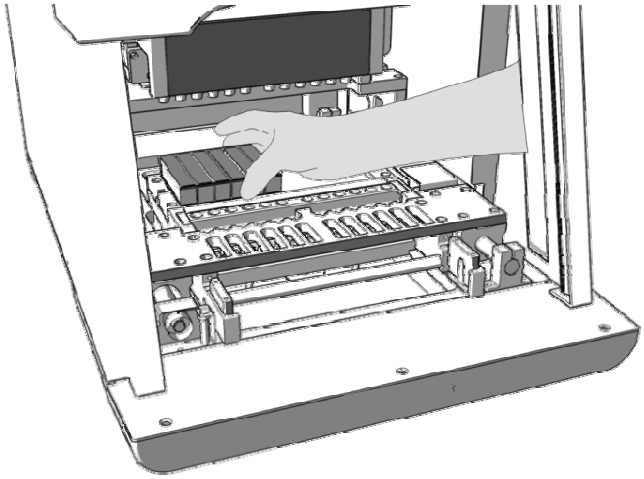
In accordance with BioChain's ISO-certified Quality Management System, each lot of the AnaPrep FFPE DNA Extraction Kit is tested to ensure consistency in product quality.

Extraction Protocol

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.

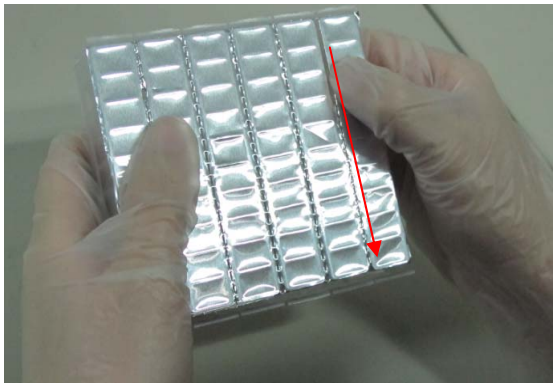
4. Load Reagent Cartridges, and all plastic disposables (Reaction Chamber, Tip Holder, Piercing Pin, and Filtered Tip).

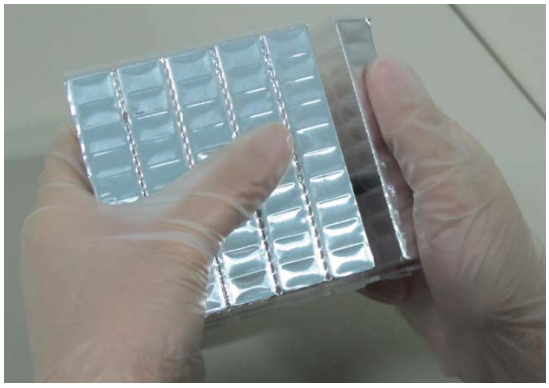


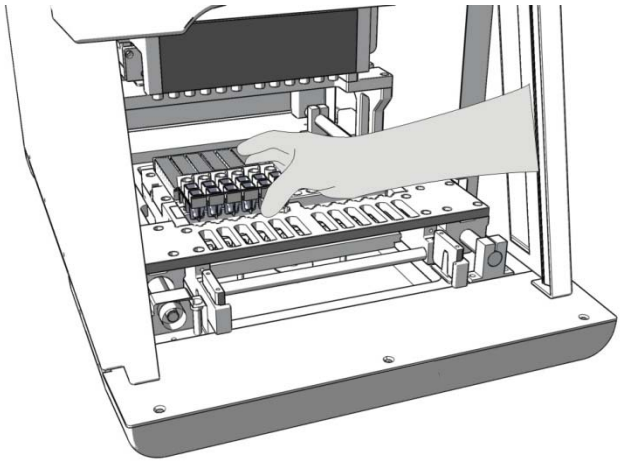
Insert Reagent Cartridges

■ How to pull apart the reagent cartridges

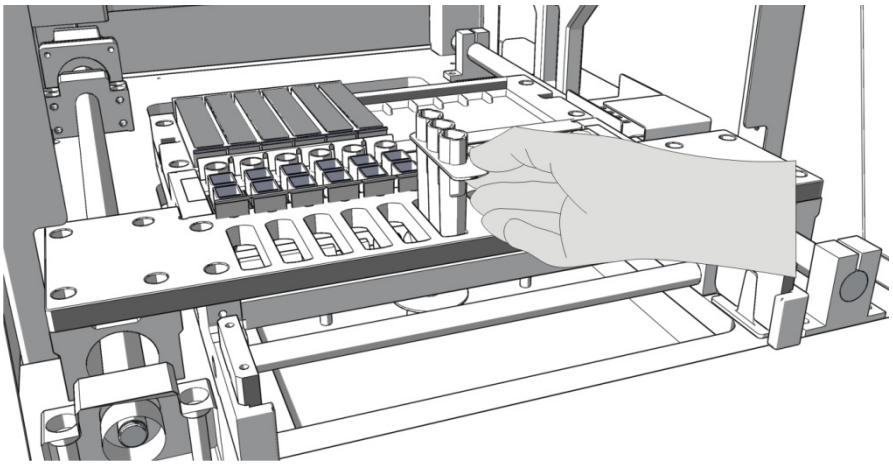
- Cut foil with a finger nail along the dotted line and then snap it apart with a little bit of force.



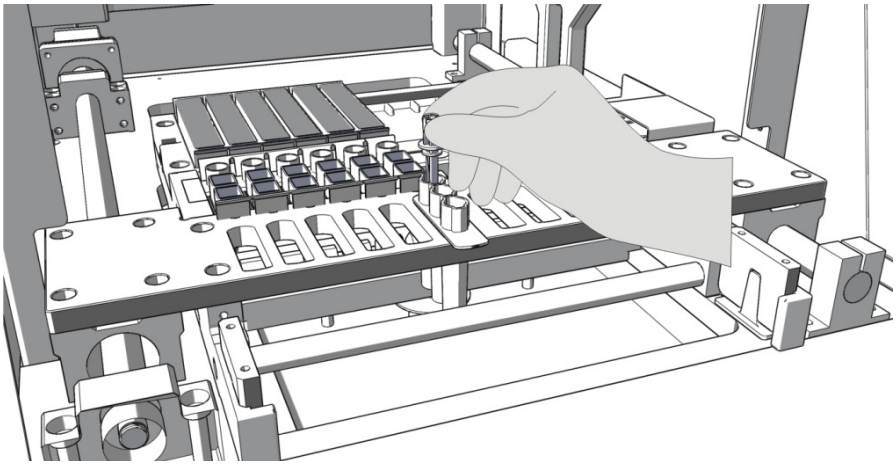




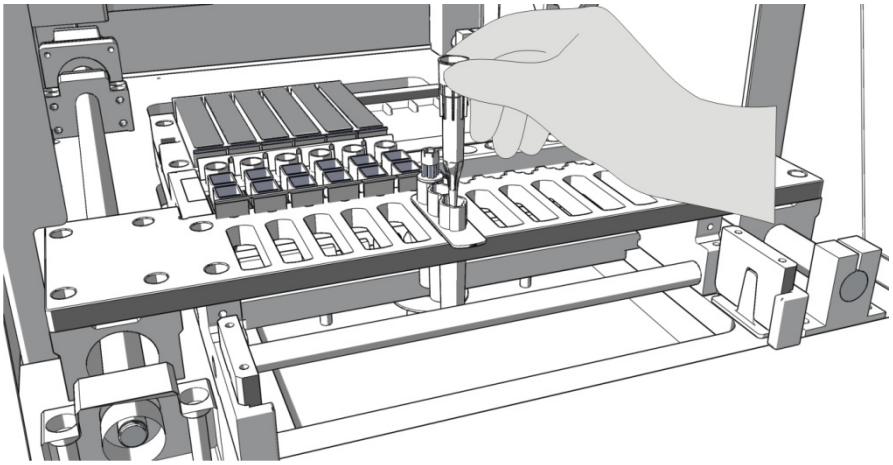
Insert Reaction Chambers



Insert Tip Holder



Insert Piercing Pins



Insert Filtered Tips

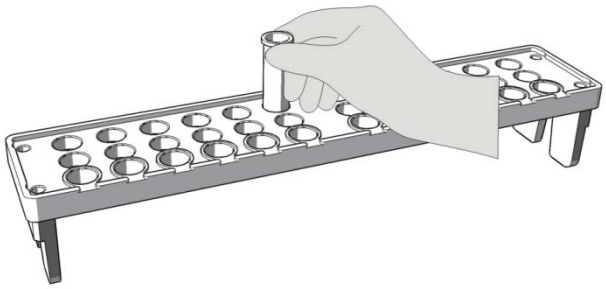
Note:

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

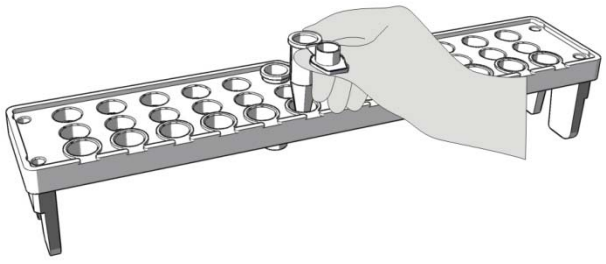
Important:

- 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

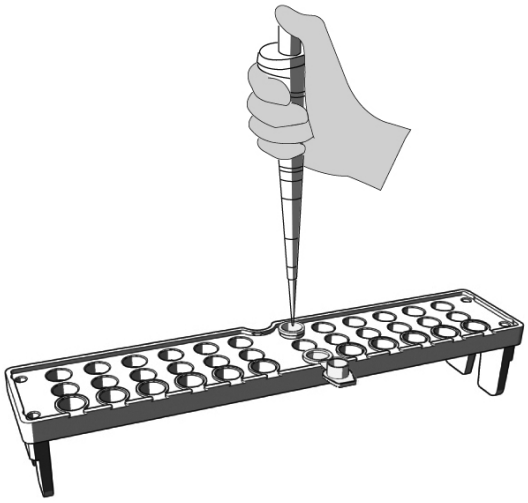


Insert Sample Tube into the Sample Rack



Insert Elute Tube into the Sample Rack

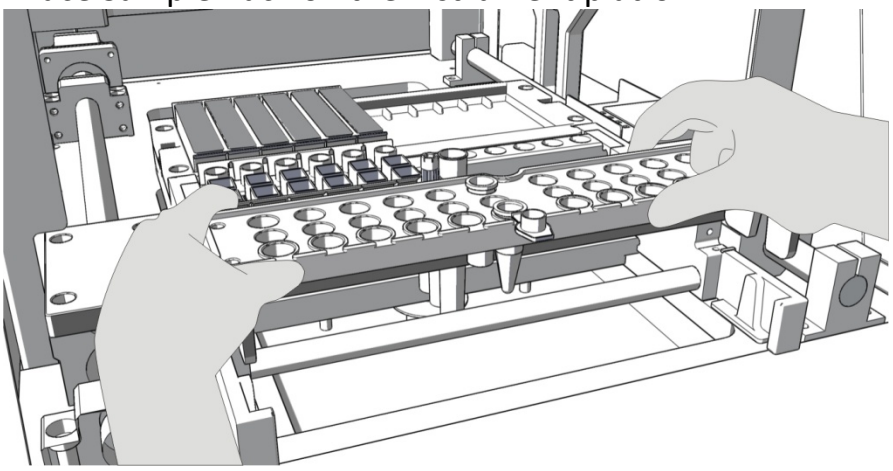
6. Load the sample(s) to Sample Tube.



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.

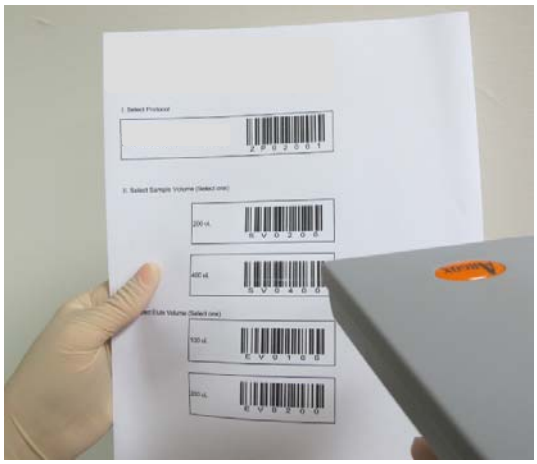
7. Place Sample Rack on the instrument platform



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^{o}C for short-term storage or store at -70^{o}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.

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