Snibe Diagnostic

CE REF 132131001HC: 32 Isolations/kit

Molecision™ Nucleic Acid Extraction Kit

INTENDED USE

The Nucleic Acid Extraction Kit is intended for isolating nucleic acids (NA) from sample materials for an *in vitro* testing.

SAMPLE MATERIALS

The sample materials that can be specifically isolated are as follows.

Target material	Sample material
Viral NA	Plasma, Serum, Whole blood, Sample preservation solution from oropharyngeal swabs and nasopharyngeal swabs
Genomic DNA	Whole blood, Cultured cells, Fresh-frozen tissue
Human cell-free NA	Plasma

The Fresh-frozen tissue should be fully grinded and mixed by liquid nitrogen.

PRINCIPLE/SUMMARY

The nucleic acid isolation procedure is based on the silica magnetic glass particles as a solid-phase support technology[1].

The main steps of this isolation procedure are:

1. Nucleic acids are released in the lysis buffer
2. Nucleic acids are bonded to silica surface of the magnetic glass particles by hydrogen bonding and electrostatic adsorption.
3. Nucleic acids are magnetically separated from the residual lysed sample.
4. Unbound substances, such as proteins, cell debris and PCR inhibitors are removed by several washing steps.
5. Elution buffer elute the purified nucleic acids from the magnetic glass particles.

REAGENT

Kit Contents

Component	Contents
Reagent Plate	2 plates
Proteinase K	320 µL, 1 vial
8-Strip Tip	4 strips

The Reagent Plate is comprised of 6 reagent containers:

Container	Component	Position	Volume	Description
1	Lysis Buffer	Column 1/7	500 µL/Well	Guanidine Thiocyanate
2	Wash Buffer I	Column 2/8	600 µL/Well	Guanidine Hydrochloride, Ethanol
3	Wash Buffer II	Column 3/9	600 µL/Well	Tris-HCl, Ethylene diamine tetraacetic acid
4	Wash Buffer III	Column 4/10	600 µL/Well	Tris-HCl, Ethylene diamine tetraacetic acid
5	Elution Buffer	Column 5/11	80 µL/Well	Tris-HCl
6	Magnetic Glass Particles	Column 6/12	150 µL/Well	Magnetic Glass Particles

The column represents the position of Reagent Plate, as shown below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Lysis Buffer	Wash Buffer I	Wash Buffer II	Wash Buffer III	Elution Buffer	Magnetic Glass Particles	Lysis Buffer	Wash Buffer I	Wash Buffer II	Wash Buffer III	Elution Buffer	Magnetic Glass Particles
B												
C												
D												
E												
F												
G												
H												

WARNINGS AND PRECAUTIONS

- For professional use only.
- Read all the instructions carefully before using the kit.
- Do not use kit beyond the expiration date indicated on the labels.
- Do not interchange reagent components from different lots.
- Personal protective measures should be taken to prevent any part of the human body from contacting samples and reagents, and should comply with local operating requirements for them, e.g., wear protective disposable gloves, laboratory coats and eye protection.
- Do not eat, drink, or smoke in the laboratory work area and do not pipette by mouth.
- The reagents contain dangerous or hazardous composition. If the reagents touch the skin, eyes or mucous membranes, wash the affected area immediately with large amounts of water. If the reagents are spilled, dilute the spill with water before wiping it up.
- Do not allow reagents or waste containing guanidine thiocyanate to contact acids or sodium hypochlorite solution. These mixtures produce a highly toxic gas.
- Always avoid microbial and nuclease contamination of reagents once they are opened and using sterile disposable pipette tips to avoid microbial and nuclease contamination of reagents when removing aliquots from reagent containers.
- Discard pipette tips in sealed containers to prevent airborne contamination.
- Avoid touching surface of materials without wearing gloves. Change gloves frequently to handle specimens and kits, and wash hands thoroughly after handling.
- Before use, visually inspect the reagent cassettes to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for nucleic acid isolation.
- Samples must be treated as potentially infectious and/or biohazardous, as well as all the reagents and materials that have been exposed to the samples and they must be handled according to the local regulations.

MATERIALS REQUIRED (PROVIDED)

- Nucleic Acid Extraction Kit.

MATERIALS REQUIRED (NOT PROVIDED)

- Fully-auto Nucleic Acid Purification System: Molecision MP-32 and Molecision MP-96.
- Standard laboratory equipment: centrifuge for tubes, etc.
- RNase/DNase-free consumables: 1.5 mL or 2 mL tubes, pipettes, aerosol-resistant pipette tips and disposable latex or vinyl gloves.
- Optional, large package of 8-Strip Tip (REF: 132131002H) and 96-Well Deep-Well Plate (REF: 132131003H).

STORAGE AND STABILITY

Reagents

- The sealed components are stable at 2-8°C until the expiration date as stated on the labels.
- Do not freeze the kit.
- If reagents could not be used at once, transfer the reagents into RNase/DNase-free tubes .Tightly cap the tubes, store at 2-8°C up to 3 months.
- Protect from sunlight and magnets.

Sample Materials

- Use fresh sample materials stored at 2-8°C up to 24h for extraction is recommended. If not, follow the recommendation below. Store sample materials are no more than 6 months at -20±5°C, or one year at -70±5°C.
- Plasma or blood does not contain heparin, since this can negatively impact the performance of the downstream PCR.

Purified Nucleic Acids

- Use the purified nucleic acids immediately for sensitive detection, or store at 2-8°C up to 24h, or 7 days at -20±5°C, or 6 months at -70±5°C.
- In the case of freezing nucleic acids, it is recommended to save aliquots separately to minimize the freezing and thawing cycles.

PROCEDURE OF INSTRUMENT EXTRACTION

- **Preparation of Sample Materials**

Sample materials	Processing methods
Whole blood, Plasma, Serum	Prepare 300 µL fresh or frozen sample without any pretreatment. The sample material should be completely homogenized (No clots in serum, anticoagulated plasma and whole blood samples).
Sample preservation solution	Prepare 300 µL volume of sample, violent vortex oscillate for 1 min.
Fresh-frozen tissue	Add 300 µL phosphate buffered saline (1×PBS) into each sample, oscillatory mixing the sample.
Cultured cells	Centrifuge appropriate volume of sample at 300×g for 5min to concentrate cells into pellet. Discard supernatant and re-suspend the pellet in 300 µL 1×PBS.

If sample volume is less than 300 µL, add an appropriate volume of 1×PBS to make total volume up to 300 µL.

For cultured cells, not more than 5×10^6 cells/300 µL, excess cells may reduce extraction efficiency.

- **Preparation of Reagent**

Take the Reagent Plate out and equilibrate to room temperature. Turn upside down three times, remove the plastic film and centrifuge briefly in a 96-well plate centrifuge (or shake it by hand) to avoid hanging the liquid on the walls or aluminum foil film. Tear off the aluminum foil film on the Reagent Plate and confirm the direction of the plate (magnetic beads are in column 6/12).

- **Addition of Sample**

Add 300 µL liquid sample and 10 µL proteinase K to the first and seventh columns of the Reagent Plate. Take care to avoid cross-contamination.

If internal control (IC) needs to be mixed with sample, ensure that the total volume of IC and sample should not exceed 300 µL.

- **Load of Reagent Plate**

Load the reagent plate on the Fully-auto Nucleic Acid Purification System. Install two 8-Strip Tip with one plate. Molecision MP-32 install up to four 8-Strip Tips and two plates at a time, Molecision MP-96 install up to twelve 8-Strip Tips and six plates at a time.

- **Setup of Program**

According to the table to establish the extraction procedures on the instrument, or use a scanner to scan the QR code of the kit label to automatically obtain the extraction procedures, and set up the extraction procedures as a template for subsequent use.

Step	HolePos	StepNameId	WaitTime (min:s)	Mix time (min:s)	MagTime (min:s)	Adsorpt Mode	MixMode	Volume (µL)
1	1/7	Lysis	00:00	10:00	00:00	Normal	High Speed	800
2	6/12	Beads	00:00	00:15	00:30	Strong	Mid Speed	150

3	1/7	Binding	00:00	10:00	00:35	Strong	Mid Speed	800
4	2/8	Wash 1	00:00	02:00	00:30	Strong	Low Speed	600
5	3/9	Wash 2	00:00	01:00	00:30	Strong	Low Speed	600
6	4/10	Wash 3	00:00	01:00	00:30	Strong	Low Speed	600
7	5/11	Elute	02:00	05:00	00:35	Normal	High Speed	80
8	6/12	Discard	00:00	00:30	00:00	Normal	High Speed	150

Temperature of lysis is 75°C, continuous heating ends at step 2.
Temperature of elution is 75°C, continuous heating starts at step 7.

- **Nucleic Acid Storage**

When the program was auto completed, transfer the nucleic acid of Well Position 5/11 into RNase/DNase-free tubes. If nucleic acid is not used immediately, store the nucleic acid according to STORAGE AND STABILITY: Purified Nucleic Acids section.

Quality control

If the downstream application needed, add internal control in the extraction protocol.

LIMITATIONS

- The kit has been validated only for the sample materials specified in SAMPLE MATERIALS section.
- Use of this kit should be limited to personnel trained in molecular diagnostic techniques.
- False negative results may occur if components of kit are in improper conditions (temperature, time) or inadequate numbers of organisms are present in the sample materials.
- Reliable results depend on proper sample collection, transport, storage and handling procedures and other factors that have not been evaluated.
- Reliable results are dependent on downstream nucleic acid testing instrument. Any IVD application using the sample preparation procedure in conjunction with any downstream IVD nucleic acid testing shall be validated with regard to the individual IVD parameters, and any result shall be interpreted within the context of all relevant clinical and laboratory findings.
- To minimize the risk of a negative impact on the results, adequate controls for downstream applications must be used.

PERFORMANCE

Analytical Performance

Real-time qPCR/RT-PCR was performed on the Bioer LineGene 9600 Instrument using 20 µL eluate in a total PCR volume of 50 µL. Quantification control samples were added directly before starting the PCR.

Sample Type	LOD
Sample preservation solution from oropharyngeal swabs (SARS-CoV-2 Virus)	120 copies/mL
Plasma (HBV Virus)	5 IU/mL

Precision Analysis

To determine the intra-assay precision, all data were produced in a single run. To determine the inter-assay precision three runs were performed by different operators, on different instruments, in different labs. Lot-to-lot precision was determined using three different lots.

Sample Type	Intra-run precision	Inter-run precision
Sample preservation solution from oropharyngeal swabs (SARS-CoV-2 Virus)	≤3 %	≤5%
Plasma (HBV Virus)	≤3%	≤5%

Purification Efficiency

To determine the precision of the purification efficiency, genomic DNA was purified from whole blood. Yield and purity of the isolated nucleic acid were determined by OD measurement. The experimental setup and the results are described below.

Sample Type	Volume (µL)	Yield (µg)	Purity	
			OD260/280	OD260/230
Whole blood (2.3×10^3 white blood cells/mL)	100 200 300	1.1~1.3 2.0~2.1 2.7~3.0	1.8~2.0 1.8~2.1 1.9~2.0	1.5~1.7 1.5~1.7 1.6~1.8
cultured cells (5.4×10^6 cells/mL)	30 300	8.6~9.2 24.3~27.6	1.8~2.1 1.9~2.1	1.5~1.8 1.6~1.8

REFERENCES

1. Alfredo Ribeiro-Silva, et al. RNA extraction from ten year old formalin-fixed paraffin-embedded breast cancer samples: a comparison of column purification and magnetic bead-based technologies. BMC Mol Biol . 2007,8:118.

ADDITIONAL INFORMATION

SYMBOLS EXPLANATIONS

Consult instructions for use		Manufacturer	
8℃ 2℃	Temperature limit (Store at 2-8°C)		Use-by date
Σ	Contains sufficient for<n> tests		Keep away from sunlight
	This way up	LOT	Batch code
IVD	*In vitro* diagnostic medical device	EC REP	Authorized representative in the European Community
REF	Catalogue number	CE	CE marking

Shenzhen New Industries Biomedical Engineering Co., Ltd.
No.23, Jinxiu East Road, Pingshan District, 518122 Shenzhen, P.R. China
Tel: +86-755-21536601 Fax: +86-755-28292740

EC REP **Shanghai International Holding Corp. GmbH (Europe)**
Eiffestrasse 80, 20537 Hamburg, Germany
Tel: +49-40-2513175 Fax: +49-40-255726