IVD CE

Instruction of RNA/DNA Purification Kit (Magnetic Bead)

[Product Name]

Generic name: RNA/DNA Purification Kit (Magnetic Bead)

[Package Specifications]

Large package: 20 tests/kit; 96 tests/kit

Pre-packaged: 20 tests/kit; 32 tests/kit

Single pre-packaged: 32 tests/kit

[Intended Use]

This kit is used for extraction, enrichment, and purification of nucleic acid. Products processed by the kit are used for clinical in vitro detection.

[Test Principle]

Magnetic bead binding liquid contains powerful protein denaturation agent, which can rapidly dissolve proteins and dissociate nucleic acid. In its presence, released nucleic acid components can bind to magnetic beads; Then, by the action of magnetic bead washing solution 1 and magnetic bead washing solution 2, the protein, inorganic salt ions, and many organic impurities were removed, and then the pure nucleic acid was eluted by the eluent.

[Main Components]

Large package: 20 tests/kit

Component name	Specification	Quantity
Magnetic beads	500 μL/tube	1
Magnetic bead binding liquid (concentrate)	7 mL/ bottle	1
Magnetic bead washing solution 1 (concentrate)	6.3 mL/ bottle	1
Magnetic bead washing solution 2	13 mL/ bottle	1
Eluent	3 mL/ bottle	1
Protease K	500 μL/ tube	1

Large package: 96 tests/kit

Component name	Specification	Quantity
Magnetic beads	2.2 mL/ tube	1
Magnetic bead binding liquid (concentrate)	32.5 mL/ bottle	1
Magnetic bead washing solution 1 (concentrate)	27 mL/ bottle	1

Magnetic bead washing solution 2	60 mL/ bottle	1
Eluent	15 mL/ bottle	1
Protease K	1.1 mL/ tube	2

Pre-packaged: 20 tests/kit;

Component name	Specification	Quantity
Pre-packaged 96-well plate	-	2
Protease K	500 μL/tube	1

Pre-packaged:32 tests/kit

Component name	Specification	Quantity
Pre-packaged 96-well plate	-	2
Protease K	800 μL/tube	1

Single pre-packaged: 32 tests/kit

Component name	Specification	Quantity
Single extraction plate of pre-packaged nucleic acid extraction reagent	-	32 pieces
Protease K	800μL/tube	1

Self prepare reagents: Anhydrous ethanol, isopropanol

Note: Components in different specification or different lots of kits cannot be interchangeable.

If the magnetic bead binding solution (concentrate) or magnetic bead washing solution 1 (concentrate) in large package specification has crystallization, please preheat at 58 ℃ and use after fully dissolving.

[Storage Conditions and Validity Date]

Store at room temperature, and the validity period is 12 months.

[Applicable Instruments]

Automatic nucleic acid extraction instrument: Smart32, Stream SP96, Ballet X3, DA3200, DA3300, DA3500, Kingfisher96, and other same type instruments.

[Sample Requirements]

1. Sample type: serum, plasma, cervical exfoliated cells, throat swab, nasopharyngeal secretion, sputum, bronchoalveolar lavage fluid and etc.

2. Sample collection

2.1 Serum: Draw 2 mL venous blood of the to be tested personnel with a disposable sterile syringe, inject it into a sterile dry glass tube, and place it at room temperature (22-25 ℃) for 30 to 60 minutes. The serum can be obtained after the blood samples agglutinate and precipitate spontaneously, or by directly centrifuged at 1500 rpm for 5 minutes in a horizontal centrifuge. Draw the serum in upper layer, and transfer it to a 1.5 mL sterilized centrifuge tube.

2.2 Plasma: Draw 2 mL venous blood of the to be tested personnel with a disposable sterile syringe, inject it into a glass tube containing EDTA (Ethylenediaminetetraacetic acid disodium) or sodium citrate anticoagulant. Gently reverse the glass tube and mix for 5-10 times, so that the anticoagulant and venous blood are well mixed. The plasma could be separated after 5-10 minutes, then transfer it to a 1.5 mL sterilized centrifuge tube.

2.3 Handling of samples like throat swab, nasopharyngeal secretion, and etc., please refer to the monitoring plan and the detection plan for the detection object issued by Ministry of Health.

2.4 Collection of cervical exfoliated cells should be performed by medical personnel using vaginal speculum or vaginal opener to expose the cervix first, and then place the cervical brush in the cervix, rotate clockwise for 4-6 rounds to access adequate epithelial cell, take out the cervical brush and put it into a sterile test tube containing 2 mL of saline.

2.5 Sputum: The natural coughed sputum or the sputum sucked by disposable sputum aspirator is treated with sample diluent (such as 4% sodium hydroxide or saline) for liquefaction, then ready for use.

2.6 Bronchoalveolar lavage fluid: Clinician collects samples according to corresponding operation procedure. Use bronchoscope to inject saline into the bronchoalveolar and then exhaled into a sterile bottle.

3. Sample Storage and transportation: the sample can be used for detection immediately, or to be stored at -20 ℃ waiting for detection. The storage period of sample should be determined according to regulations of the PCR kit used. The sample should be transported using 0℃ curling.

[Handling of Sample]

1. Pre-treatment of sputum sample

Add 4% NaOH solution with 4 times the volume of the sputum, shake well and place at room temperature for about 30 minutes for liquidation. Take 0.5 mL to 1.5 mL of above solution to a centrifuge tube, then add 0.5 mL of 4% NaOH solution and place it at room temperature for 10 minutes to fully liquefy (no obvious solid and no pulling off phenomenon when it is sucked out is considered as complete liquidation). Centrifuge at 13,000 rpm/min, for 5 min. Carefully absorb and dispose 800 μL of supernatant, take the 200 μL of sample at the bottom, it can be used for nucleic acid extraction after mix well.

2. Pre-treatment of bronchoalveolar lavage fluid

Take 1 mL to 1.5 mL of bronchoalveolar lavage solution to a 1.5 mL centrifuge tube, centrifuge at 13,000 rpm/min for 5 min. Carefully absorb and dispose 800 μL of supernatant, take the 200 μL of sample at the bottom, it can be used for nucleic acid extraction after mix well.

[Test Method]

1. Preparation before experiment

1.1 Large package: 20 tests/kit

1.1.1 Add 3.8 mL of isopropanol into the magnetic bead binding liquid (concentrate), and tick on the tube cap and tube

wall. Store it at room temperature.

1.1.2 Add 7.7 mL of anhydrous ethanol into the magnetic bead washing solution 1 (concentrate), and tick on the tube cap and tube wall. Store it at room temperature.

1.2 Large package: 96 tests/kit

1.2.1 Add 17.5 mL of isopropanol into the magnetic bead binding liquid (concentrate), and tick on the tube cap and tube wall. Store it at room temperature.

1.2.2 Add 33 mL of anhydrous ethanol into the magnetic bead washing solution 1 (concentrate), and tick on the tube cap and tube wall. Store it at room temperature.

Note: If the magnetic bead binding liquid and the magnetic bead washing solution 1 were improperly placed at low temperature, crystalline precipitate may appear, put it into 37 ℃ warm bath until it disappears.

After using each reagent, please tighten the bottle cap.

2. Extraction of nucleic acid

Operation of automatic nucleic acid extraction instrument: Smart32

I Large package: 20 tests/kit or 96 tests/kit

Preparation of deep-well plate

Two 96-deep-well plates can be process in one experiment by one Smart32 nucleic acid extraction instrument, and 16 samples can be extracted in one 96-deep-well plates each time.

① According to the quantity of extraction samples, add 600 μL of magnetic bead washing solution 1 to the third and ninth column of the 96-deep-well plates.

② According to the quantity of extraction samples, add 600 μL of magnetic bead washing solution 2 to the fourth and tenth column of the 96-deep-well plates.

③ According to the quantity of extraction samples, add 100 μL of eluent to the sixth and twelfth column of the 96-deep-well plates.

④ According to the quantity of extraction samples, add 20 μL of protease K, 20 μL of magnetic beads, 200 μL of sample, and 500 μL of magnetic bead binding liquid to the first and seventh column of the 96-deep-well plates.

Note: 1. Magnetic beads should be thoroughly mixed before adding to the plate. If the quantity of samples to be extracted is large, it is recommended to re-suspend the beads once every 8 sample holes are added.

2. The second, fifth, eighth and eleventh column of the plate do not participate in the extraction process, no reagents should be added to these columns.

Program setting

Step	Well	Name	Waiting time (Min)	Mixing time (Min)	Magnetic binding times	Mixing velocity	Volume (μL)	Temperature status	Temperature (℃)
1	1	Magnetic bead binding liquid	0	15	3	Fast	750	Well 1	70
2	3	Magnetic bead washing solution 1	0	2	2	Fast	600	Well 6	50
3	4	Magnetic bead washing solution 2	0	0	1	Medium	600	Well 6	70
4	6	Eluent	0	5	3	Medium	100	Well 6	70
5	3	Discard the magnetic beads	0	1	0	Medium	600	Close	0

After the program is finished, the liquid in the sixth and twelfth column is nucleic acid liquid, it is recommended to be used immediately. If storage of the liquid is required, please transfer it into sterile centrifuge tube, and store at -20℃.

II Pre-packaged: 20 tests/kit

Preparation of deep-well plate

① Take out the pre-packaged 96-deep-well plate from the kit, reverse to blend for several times, shake the plate gently to gather the reagents and magnetic beads to the plate bottom, (or centrifuge at 500 rpm for 1 minute by 96-well plate centrifuge machine), before use carefully tear off the sealing film to prevent the liquid from splashing out.

② Add 200 μL of sample and 20 μL of protease K to the first and seventh column (of A-E row) of the 96-deep-well plate in order.

Program setting

Step	Well	Name	Waiting time (Min)	Mixing time (Min)	Magnetic binding times	Mixing velocity	Volume (μL)	Temperature status	Temperature (℃)
1	2	Magnetic beads	0	1	3	Medium	400	Close	0
2	1	Magnetic bead binding liquid	0	15	3	Fast	750	Well 1	70
3	3	Magnetic bead washing solution 1	0	2	2	Fast	600	Well 6	50

4	4	Magnetic bead washing solution 2	0	0	1	Medium	600	Well 6	70
5	6	Eluent	0	5	3	Medium	100	Well 6	70
6	3	Discard the magnetic beads	0	1	0	Medium	600	Close	0

After the program is finished, the liquid in the sixth and twelfth column is nucleic acid liquid, it is recommended to be used immediately. If storage of the liquid is required, please transfer it into sterile centrifuge tube, and store at -20 ℃.

III Pre-packaged: 32 tests/kit

Preparation of deep-well plate

① Take out the pre-packaged 96-deep-well plate from the kit, reverse to blend for several times, shake the plate gently to gather the reagents and magnetic beads to the plate bottom, (or centrifuge at 500 rpm for 1 minute by 96-well plate centrifuge machine), before use carefully tear off the sealing film to prevent the liquid from splashing out.

② Add 200 μL of sample and 20μL of protease K to the first and seventh column (of A-H row) of the 96-deep-well plate in order

Program setting: The same as the program of pre-packaged: 20 tests/kit.

After the program is finished, the liquid in the sixth and twelfth column is nucleic acid liquid, it is recommended to be used immediately. If storage of the liquid is required, please transfer it into sterile centrifuge tube, and store at -20 ℃.

IV Single pre-packaged: 32 tests/kit

Preparation of deep-well plate

① Take out the nucleic acid extraction reagent from the kit, reverse to blend for several times, shake the extraction tube gently to gather the reagents and magnetic beads to the tube bottom, (or place the single extraction plate on single extraction plate holder, and centrifuge at 500 rpm for 1 minute by 96-well plate centrifuge machine), before use carefully tear off the sealing film to prevent the liquid from splashing out.

② Add 200 μL of sample and 20 μL of protease K to the first well of the single extraction plate in order

③ Place the single extraction plate on the holder properly, and then put it on extraction instrument for extraction.

Program setting: the same as pre-packaged: 20 tests/kit.

After the program is finished, the liquid in the sixth well is nucleic acid liquid, it is recommended to be used immediately. If storage of the liquid is required, please transfer it into sterile centrifuge tube, and store at -20 ℃±5 ℃.

2. Operation of automatic nucleic acid extraction instrument: Stream SP96

2.1 Startup the power of automatic nucleic acid extraction instrument: Stream SP96.

2.2 Startup the computer connected to the instrument, click the software icon of Stream SP96, enter the operation

interface of automatic nucleic acid extraction instrument: Stream SP96.

2.3 After entering the software operation interface, initialize the instrument, click icon of “New experiment”, scan the sample code, select “Project”, select “Protocol”, enter “Experiment No.”, and then select quantity of samples and quality controls.

2.4 The software interface point out the placement of reagents and corresponding consumables.

2.5 Open the instrument cabin door, place corresponding experiment materials strictly according to the hint of software. (Notice: There should be no impurities, clotting, bubbles, or hanging droplets in the sample. Unqualified samples may lead to wrong detection result. Avoid bubbles and precipitates when loading reagents. When loading the gun head, the gun head box must be pushed into the slot along the head card slot to ensure the gun head box is placed in place.)

2.6 Check whether the placement of consumables and reagents are correct.

2.7 Close the instrument cabin door. Click the "run" button on the software to start the experimental program.

2.8 After the program is finished, the nucleic acid in the eluting well can be used for amplification detection.

2.9 One tube of negative control and one tube of positive control should be processed at the same time with the sample, and the process should be the same as that of sample.

[Limitations of Product]

The efficiency of sample extraction is related to whether the operator strictly follows the operation instructions. If the cross-contamination is not well controlled during sample processing, false positive results may occur.

[Product Performance Indicators]

1. This kit can reach the same performance indicators of the same reagent at home and abroad
2. This kit can be used for nucleic acid extraction of different types of samples, the extraction efficiency of low concentration samples is better.
3. This kit has good repeatability, and the intra-batch CV value and inter-batch CV value are all less than 5%.

[Precautions]

1. Please read the Instructions for Use carefully before experiment.
2. **Different extraction program may need to set up due to the hardware difference.** Please consult our company for detailed parameter setting.
3. In order to avoid any potential biological hazards in the samples, the test samples should be regarded as infectious and avoid contact with human skin and mucosa; the samples should be handled in a biosafety cabinet that prevents aerosol outflow. The test tubes and tips used in the sample preparation area should be poured into a container containing disinfectant and sterilized with the medical wastes before discarding; sample handling and processing must comply with relevant regulations: including the General Biosafety Standard for Microbiological and Biomedical Laboratories and Regulations on the Administration of Medical Wastes issued by the Ministry of Health.

4. The components in the kit should be used within the validity period. Experiment without the components provided in this kit may lead to wrong results.

5. The laboratory management shall be in strict accordance with the management practice of PCR gene amplification laboratory. The laboratory personnel must receive professional training. The experimental process shall be strictly divided into different areas (reagent preparation area, sample preparation area, amplification test area). All consumables shall be sterilized for single use. Special instruments and equipment shall be used in each stage of experiment. No cross-utilization of the supplies of each area in each stage shall be allowed.

6. Use autoclaved disposable centrifuge tubes and suction heads or purchase of DNA/RNA enzyme-free centrifuge tubes and suction heads.

7. After the RNA sample extraction is completed, it is recommended to proceed to the next experiment immediately; otherwise, please store the extract at -20 ℃ for use (within 24h).

8. After the experiment, 10% hypochlorite or 75% alcohol should be used to disinfect the worktable and pipette, followed by exposing them in ultraviolet light for 20-30 minutes.

[References]

1. Vogelstein B , *et al*. Preparative and analytical purification of DNA from agarose. *Proc Natl Acad Sci,*1979, 76 (2), 615-619.

2. Shale Dames , *et al*. A Single-Tube Nucleic Acid Extraction, Amplification,and Detection Method Using Aluminum Oxide. *Journal of Molecular Diagnostics,*2003*,* 8(1),16-21.

3. Grieg F , *et al*. Extraction and purification of nucleic acids from viruses. *MAVE Chapter,* 2010, 154–165.

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[Index of CE Symbols]

IVD	For in vitro diagnostic use only		Do not reuse
	Expiry date		See instruction for use
	Warning, please refer to the instructions in the annex		Manufacturer
	Temperature scope within which the product is reserved	LOT	Batch number
REF	Catalog #		Tests per kit
EC REP	European union authorized representative		
	Keep away from sunlight		Biological risks
CE	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC		

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