IVD CE

Instruction of RNA/DNA Purification Kit (Spin Column)

[Product Name]

Generic name: RNA/DNA Purification Kit (Spin Column)

[Packaging Specification] 20 tests/kit; 48 tests/kit.

[Intended Application] This kit is used for extraction, enrichment, purification, etc. of nucleic acid. Products processed by the kit are used for clinical in vitro detection.

[Testing Principle]

Lysis solution contains strong protein denaturant, which can quickly dissolve protein and make nucleic acid dissociated; under the existence of lysis solution and ethyl alcohol, the dissociated nucleic acid compositions can be combined on the silicone membrane; then by actions of inhibitor remover and deionized solution, remove the protein, inorganic salt ions and many organic impurities. Then use eluent to elute pure nucleic acid.

[Main Components]

20 tests/kit

Component name	Specification	Quantity
Spin column	20/bag	1
Collection-tube	80/bag	1
Lysis solution	4.4 mL/bottle	1
Inhibitor remover (concentrated solution)	7 mL/bottle	1
Deionized solution (concentrated solution)	4.4 mL/bottle	1
Eluent	3 mL/bottle	1
Proteinase K	1.2 mL/bottle	1
Carrier RNA (dry powder)	155 μg/tube	1

48 tests/kit

Component name	Specification	Quantity
Spin column	48/bag	1
Collection-tube	96/bag	2
Lysis solution	11 mL/bottle	1
Inhibitor remover (concentrated solution)	16.5 mL/bottle	1

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Deionized solution (concentrated solution)	11 mL/bottle	1
Eluent	6 mL/bottle	1
Proteinase K	2.7 mL/bottle	1
Carrier RNA (dry powder)	155 μg/tube	2

Self prepare reagents: absolute ethyl alcohol.

[Storage Conditions and Validity Date]

Preserve it under room temperature with a period of validity of 12 months. Carrier RNA shall be stored at -20℃±5℃ after dissolution, repeated freezing and thawing are avoided; the rest components of nucleic acid extraction reagent are stored at room temperature. See the product label for the manufacture date and expiry date of the reagent kit.

[Applicable Instrument] Centrifugal machine up to 12,000g.

[Samples Requirements]

1. Applicable specimen type: Serum, plasma, cervical exfoliated cell, throat swab, nasopharyngeal secretions, etc.

2. Specimen collection

2.1 Serum - using disposable sterile syringe drawing 2 mL venous blood of patient, injecting into sterile dry glass tube, room temperature (22~25°C) placing 30~60 min, blood specimen can be spontaneous completely agglutination separate out serum, or directly using horizontal centrifuge, 1,500g centrifuging 5 min). Take the upper serum and transfer to a 1.5 mL sterilization centrifuge tube.

2.2 Plasma - using disposable sterile syringe drawing 2 mL venous blood of patient, injecting into glass tube with EDTA (Ethylenediaminetetraacetic acid disodium) or Sodium Citrate Injection for Anticoagulant, immediately gently reverse glass tube 5~10 times, making the anticoagulation and venous blood sufficient mixed, after 5~10 minutes can be isolated plasma, transferring to 1.5 mL sterilization centrifuge tube.

2.3 For treatments on samples of throat swab, nasopharyngeal secretions, etc., please refer to the monitoring scheme and detecting technique scheme on corresponding detected matter issued by Ministry of Health.

2.4 For collection of specimens of cervical exfoliated cells, firstly, medical staff use vaginal speculum to make cervix exposed, put cervical brush on cervical mouth, swing clockwise for 4-6 turns to get sufficient epithelial cells, then take out cervical brush and put it in small sterile tube containing 2 mL of normal saline.

3. Specimen storage and transportation: The specimen can be immediately applied to test, also can be stored at -20℃ for testing. Shelf life is based on stipulated time by PCR kit. Specimen transportation adopts ice pots of 0℃.

[Usage]

1. The preparation before experiment

1.1 20 tests/kit

1.1.1 Add 4.3 mL absolute ethyl alcohol in inhibitor remover (concentrated solution) and check on the bottle cap and

body. Preserve it under room temperature.

1.1.2 Add 17.6 mL absolute ethyl alcohol in each bottle deionized solution (concentrated solution) and check on the tube cap and wall. Preserve it under room temperature.

1.1.3 Add 110 μL eluent or internal standard solution (composition in PCR kit) in Carrier RNA (dry powder), sufficient mixed, dissolved. Stored at -20℃. (**As Carrier RNA is white or translucent matter, please check carefully to ensure it completely dissolved)**

1.2 48 tests/kit

1.2.1 Add 10 mL absolute ethyl alcohol in inhibitor remover (concentrated solution) and check on the bottle cap and body. Preserve it under room temperature.

1.2.2 Add 44 mL absolute ethyl alcohol in each bottle deionized solution (concentrated solution) and check on the tube cap and wall. Preserve it under room temperature.

1.2.3 Add 110 μL eluent or internal standard solution (composition in PCR kit) in Carrier RNA (dry powder), sufficient mixed, dissolved. Stored at -20℃. **(As Carrier RNA is white or translucent matter, please check carefully to ensure it completely dissolved)**

1.3 Lysis solution and Carrier RNA mixing

Before conducting the test, lysis solution must be mixed with Carrier RNA (being arranged and utilized immediately) to be configured into lysis working solution. Its proportions are shown in following table:

	1 test	20 tests	24 tests	48 tests
Lysis solution	200 μL	4 mL	4.8 mL	9.6 mL
Carrier RNA	4 μL	80 μL	96 μL	192 μL

Note: a. If lysis solution and inhibitor remover are placed improperly under low temperature, crystalline deposit may appear. Incubate it at 37℃ until the deposit disappears; after the use of each reagent, please screw the bottle cap.

b. If there is internal standard solution provided in PCR detection reagent, use such internal standard solution to dissolve Carrier RNA (dry powder); if there is no internal standard solution in PCR detection reagent, use eluent to dissolve Carrier RNA (dry powder).

2 Sample treatment and nucleic acid extraction

2.1. Liquid specimen treatment

2.1.1 Add 50 μL of proteinase K into sterile centrifuge tube of 1.5 mL.

2.1.2 Take 200 μL specimen and add it into the centrifuge tube.

2.1.3 Add 200 μL lysis working solution (i.e. lysis solution containing Carrier RNA) , fasten down the tube cover, oscillate it in vortex for 15 seconds to mix the solution sufficiently, then conduct **high-speed centrifugation for 10 seconds** (prevent bubbles produced during incubation period) , 10 minutes at temperature of 72℃; at the same time, preheat the eluent with temperature of 72℃.

2.1.4 Add 250 μL ethanol, fasten down the tube cover. Oscillate it in vortex for 15 seconds.

2.1.5 Draw the whole mixture into the spin column, conduct 12,000g centrifugation for 1 minute at room temperature, then fit the spin column in a new collection tube.

2.1.6 Add 500 μL inhibitor remover into the spin column, conduct 12,000g centrifugation for 1 minute at room temperature, then fit the spin column in a new collection tube.

2.1.7 Add 500 μL deionized solution into the spin column, conduct 12,000g centrifugation for 1 minute at room temperature, then fit the spin column in a new collection tube.

2.1.8 Add 500 μL deionized solution into the spin column once again, conduct 12,000g centrifugation for 1 minute at room temperature, then fit the spin column in a new collection tube.

2.1.9 Place the spin column & collection-tube at room temperature, conduct 14,000g centrifugation for 3 minutes in order to remove residual ethanol.

2.1.10 Take out the spin column, fit it in a new 1.5 mL centrifuge tube. Open the cover of spin column, **lay it aside for 2 minutes at temperature of 72℃** (use dry thermostat, shall not use water bath kettle).

2.1.11 Carefully add 50 μL eluent preheated at 72℃ right above the membrane of spin column, fasten down the tube cover, after standing for 1 minute at room temperature, conduct 14,000g centrifugation for 1 minute. Then the solution in the centrifuge tube is nucleic acid solution. It is better to use it immediately, if you want to preserve it, put it at temperature of - 20 ℃.

2.2 Treatment on swab-type specimens such as throat swab

Specimens such as cervical brush, throat swab, etc. must be fully stirred up in the transporting (preserving) solution (at least for 40 times) to elute cells adhering to swabs, then take 1-1.5 mL preserving solution into 1.5 mL centrifuge tube and conduct centrifugation at 12,000 g for 3 minutes to remove most of the supernatant, reserve about 200 μL liquid with sufficient mixing, and subsequent processes are as operations in 2.1.

[Limitations of Product] Extraction efficiency of specimens is related to whether the operator is in strict accordance with the instructions. If not controlling the cross-contamination well in sample processing, a false positive result may be occurred.

[Performance Indexes of Product] Taking artificial pseudovirus PSV as detected sample, using this product can reach the same performance indexes with imported reagent of similar type (Rocher High Pure Viral Nucleic Acid Kit).

[Precautions]

1. Please read the instruction carefully before experiment.

2. In order to avoid any potential biological risk in the sample, the detected sample shall be deems as having infectious substances, avoid contacting with skin and mucos; conducting the sample treatment in biosafety cabinet which can prevent aerial fog from outflow, the test tube and pipettor used in sample preparation area shall throw into the container with sanitizer, and can be discarded after being sterilized with wastes; the sample operation and treatment shall both

conform to the requirements of relevant laws and regulations: *General biosafety standard for microbiological and biomedical laboratories* and *Regulations on the management of medical waste* issued *by* Ministry of Health.

3. The components in the kit shall be used within the period of validity, conducting experiment without using components supplied by this kit might lead to the wrong result.

4. Laboratory management shall be strictly in accordance with management standard of PCR gene amplification laboratory, the experimenter shall undergo professional training, experimental process shall perform under strict partitions (reagent preparation area, samples preparation area, amplification and product analyzing area), consumables used shall be disposable after sterilization, each stage of experimental operation shall use exclusive instrument and equipment, articles of each area and each stage shall not be of cross using.

5. Use disposable centrifuge tube and pipettor after autoclaved sterilization or purchasing centrifuge tube and pipettor without RNA enzyme.

6. After complete sample nucleic acid extraction, it is suggested to perform next experiment, otherwise please preserving in -20 °C for use (within 24 hours).

7. It shall use 10% hypochlorous acid or 75% alcohol to dispose the bench and pipettor, then use ultraviolet radiator irradiating 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. Mackay IM et al. Molecular assays for detection of human metapneumovirus. Journal of Clinical Microbiology, 2003, 41(1), 100-105.

3. Greenberger S et al. Transcription-controlled gene therapy against tumor angiogenesis. J Clin Invest, 2004,113 (7), 1017-1024.

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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 #	Σ	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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