RNA Extraction Kit

CE

REF CAMR01-05（48 Reactions）

PRODUCT NAME
RNA Extraction Kit

INTENDED USE
RNA Extraction Kit is an in vitro diagnostic test for extracting viral RNA from bronchoalveolar lavage (BAL), sputum, plasma, and cerebrospinal fluid (CSF) samples. This product is intended to be used by laboratory professional workers.

PRINCIPLE
This RNA Extraction Kit extracts RNA of pathogenic microorganisms from samples of bronchoalveolar lavage (BAL), sputum, plasma, and cerebrospinal fluid (CSF) using a centrifuge column method. Under the action of Lysis Buffer RL, RNA is fully released, and nucleic acid is adsorbed on a silica gel membrane. Then, the required RNA is purified through RNase-Free ddH_2O.

STORAGE AND STABILITY
- All reagents should be stored at room temperature（15-25℃）.
- The shelf life is 12 months.
- Production date and expiration date are shown in the package label.
- Carrier RNA lyophilized powder can be stored at room temperature (15–25°C) until the expiry date. Dissolve Carrier RNA in RNase-Free ddH_2O, then add the Carrier RNA solution to the Lysis Buffer RL and mix thoroughly (Refer to the reagent preparation steps. The Carrier RNA which dissolved in RNase-Free ddH_2O can be stored at -20°C. After adding the Carrier RNA to the Lysis Buffer RL, it can be stored at 2-8°C for no more than 48 hours, please use it immediately after preparation).

REAGENT AND MATERIALS PROVIDED

Component	Specification	Quantity
Lysis Buffer RL	30mL/bottle	2 bottles
Wash Buffer RB	13mL/bottle	1 bottle
Wash Buffer RW	12mL/bottle	1 bottle
RNase-Free ddH_2O	15mL/bottle	1 bottle
Carrier RNA	310 μg/tube	2 tubes
DNA Magnetic Beads	1.5mL/tube	1 tube
RNase-Free ddH_2O	1mL/tube	1 tube
RNase-Free Spin Column	——	50 sets
RNase-Free Centrifuge Tubes	——	50 tubes

Note: Kits with different batch numbers cannot be mixed for use.

SPECIMEN COLLECTION AND REQUIREMENTS
1. Sample collection: collect bronchoalveolar lavage (BAL), sputum, plasma, and cerebrospinal fluid (CSF) according to the specimen collection guideline, perform aseptic operation, and place the collected samples in sterile tubes. Avoid contamination during the sample collection, storage and transportation.

2. Sample storage and transportation: it is recommended to test within 12 hours after sample collection. If it cannot be tested in time, store the sample at -18°C or below for up to 1 month, at -70°C for below for up to 1 year. Samples should be shipped with dry ice. Freezing and thawing times should not exceed 3 times. Frozen sample should be thawed at room temperature before testing and mixed thoroughly before use.

3. Nucleic acid storage: Follow-up tests should be performed immediately after nucleic acid extraction.If Follow-up testscannot be performed in time, the nucleic acid should be stored at 2°C-8°C, and the follow-up tests should be performed within 12 hours, or stored at -18°C or below up to 1 month.

4. Sample safety: all samples are regarded as potentially infectious items, and the operation should be performed in accordance with relevant national standards.

TEST PROCEDURE
Refer to manual before the test. Confirm that the reagent kit is not open or broken before use, and the test should be performed at a temperature between 2-30°C.

Step 1. Sample preparation:

1.1 Sputum

For sputum and bronchoalveolar lavage (BAL) with viscous material, it needs to be liquefied before extraction.

Sample liquefaction agent made by Fuzhou Angemic Biotechnology Co., Ltd., should be performed with the following steps:

(1) Prepare the liquefaction solution based on the N+2 samples. N is the numbers of the samples to be liquefied. Mixed solution A and solution B at a volume ratio of 1:1.

(2) Samples: AB mixed solution = 1:1.5 (the sample is general 500μL for each). Then add the samples and AB mixed solution to the centrifuge tube in sequence.

(3) Wrap the tube cap with a sealing film, lay the centrifuge tube flat and fix it on the shaker, and shake it at the highest speed for 10-30 minutes until the viscous material is completely fluid (the tip will not be blocked when absorbing liquid).

1.2 Other types of samples do not require pretreatment.

Step 2. Reagent preparation:

(1) After opening the test kit, add the ethanol to Wash Buffer RB and Wash Buffer RW with the according volume on the label (17mL ethanol to Wash Buffer RB, 48mL ethanol to Wash Buffer RW).

(2) Carrier RNA solution preparation: Add 310 μL RNase-Free ddH_2O to the tube containing 310 μg of Carrier RNA lyophilized powder, completely dissolve the Carrier RNA to obtain a solution with a final concentration of 1 μg/ μL. After dissolving, dispense 10 μL processed products to RNase-Free 1.5 ml centrifuge tube and store at -20°C. The solution should not be frozen and thawed more than 3 times.

Step 3. Extraction:

3.1 Take the above prepared 1.5 ml centrifuge tube with 10 μL Carrier RNA, add 1 mL Lysis Buffer RL, vortex to mix, and centrifuge briefly.

3.2 Add 250 μl of sample, vortex to mix, and centrifuge briefly.

Note: In order to ensure sufficient lysis, the sample and Carrier RNA solution should be thoroughly mixed.

3.3 Incubate at room temperature (15-25°C) for 10 min (during incubation, take an appropriate amount of absolute ethanol and place it in a refrigerator at 4°C for 5-10 min).

3.4 Pipette 500μl liquid in the centrifuge tube to a new 1.5ml centrifuge tube and add 400μl of absolute ethanol, vortex to mix, and centrifuge briefly.

3.5 Add 600 μL absolute ethanol to the original centrifuge tube, vortex to mix, and centrifuge briefly.

3.6 Pipette the liquid in the two tubes to the same RNase-Free Spin Column in 4 times (the spin column is placed in the tube). Pipette up to 650 μL each time, centrifuge at 8000rpm for 1min , change the collection tube, and transfer 4 times in total.

Note: If the liquid on the spin column cannot be centrifuged into the collection tube, increase the speed and extend the centrifugation time until the liquid is completely transferred into the collection tube.

3.7 Carefully open the cap of the spin column, add 500 μL of Wash Buffer RB (add absolute ethanol before use), cover the tube cap, centrifuge at 8000 rpm for 1 min, and replace the collection tube.

3.8 Carefully open the cap of the spin column, add 500 μL of Wash Buffer RW (add absolute ethanol before use), cover the tube cap, centrifuge at 8000 rpm for 1 min, and replace the collection tube.

3.9 Repeat step 3.8 once.

3.10 Put the spin column into a new collection tube and centrifuge at 14000 rpm for 3min.

3.11 Open the cap of the spin column and dry at room temperature for 3-5 min to completely dry the membrane.

Note: Residues of ethanol may affect subsequent experiments.

3.12 Put the spin column into an RNase-Free 1.5ml centrifuge tube, and add 55 μL of RNase-Free ddH_2O to the membrane center of the

spin column.
Note: The eluent needs to be added to the center of the membrane of the spin column.
3.13 Close the cap, incubate at room temperature for 5 min, centrifuge at 8000 rpm for 1 min, collect nucleic acid into a centrifuge tube, and store at -20°C.
3.14 After the above operation, use Nucleic Acid Remover to clear the laboratory.

Step 4. Nucleic acid QC:

4.1 RNA concentration: with Qubit™ dsRNA HS Assay kit.

INTERPRETATION OF TEST RESULT

(1) RNA extraction concentration and volume:
Bronchoalveolar lavage (BAL), sputum, blood samples:
Concentration: ⩾ 0.1ng/μL.
Volume: ⩾0.5ng.
Cerebrospinal fluid (CSF) samples:
No specific requirement.

The following conditions may affect the extraction volume, and the experiment should be performed after excluding the influence:
(1) Sputum and viscous bronchoalveolar lavage (BAL) samples should be liquefied before nucleic acid extraction, and the time for shaking with grinding tube can be appropriately increased.
(2) For cerebrospinal fluid (CSF) samples, the time for shaking with grinding tube can be reduced to 5 minutes, or the follow-up operation can be performed directly without shaking.
(3) Due to the sample being too viscous or the concentration of nucleic acid is high, the magnetic beads might be agglomerated during the extraction process. The magnetic beads can be properly supplemented (5-10μL magnetic beads) according to the agglomeration situation.
(4) Wash Buffer RB and Wash Buffer RW need to be added with the absolute ethanol with the right volume. Reagent preparation errors can affect extraction volume.
(5) Some operational errors may lead to unqualified test results, such as: reagents used out of the validity period, inaccurate pipette, too high room temperature, and extraction procedures mis operation, etc.

LIMITATIONS

1. This kit is only suitable for the nucleic acid extraction from bronchoalveolar lavage (BAL), sputum, plasma, and cerebrospinal fluid (CSF) samples.
2. It's only for the purpose of microbial detection. It's not suitable for other purpose of the nucleic acid extraction.

WARNINGS AND PRECAUTIONS

The warnings and precautions are included, but not limited to the following:
1. For *in vitro* diagnostic use only.
2. Refer to manual before the test.
3. Samples should avoid repeated freezing and thawing, otherwise the amount of nucleic acid extraction will be reduced. Samples can be extracted immediately or stored at 2-8°C for no more than 24 hours for testing. For long-term storage, it can be stored at -20°C or below.
4. During RNA extraction, pay special attention to prevent nucleic acid degradation. The pipettes and utensils used should be dedicated, and experimental consumables such as centrifuge tubes and pipette tips should be sterilized by autoclaving. Operators should wear powder-free gloves, masks, etc.
5. It is recommended to use the Nucleic Acid Remover to clean the laboratory after each experiment.
6. All sample pretreatment and sample extraction must be performed in a biological safety cabinet, and the operation must be in strict with relevant laboratory regulations.
7. All reagents should be stored and used following to the instructions. And the reagents should be shaken and mixed before use.
8. All samples and reagents should avoid direct contact with skin and eyes. Immediately rinse with plenty of water and seek medical treatment in time once this happens.
9. The test samples are regarded as infectious products, and the operation must be performed in accordance with the operating standards of infectious disease laboratories and pay attention to biosafety operations.
10. There may be residual magnetic beads during elution, so try to avoid aspirating magnetic beads when aspirating the sample.
11. Contact manufacture for any questions.

INDEX OF SYMBOLS

IVD	In vitro diagnostic medical device	LOT	Batch code
	Date of manufacture		Use-by date
	Do not re-use		Consult instructions for use
EC REP	Authorized representative in the European Community / European Union		Manufacturer
	Temperature limits	REF	Catalogue number
	Caution	CE	CE marking

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Revision No: 01
Date of last revision: May 17, 2023

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