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EasySC Universal RNA Purification Kit

Catalog Number: R137-1, R137-2

Table 1. Kit Components and Storage

Kit Component	R137-1 (50 preps)	R137-2 (200 preps)	Storage	Stability
Buffer RL	55 mL	220 mL	RT	The product is stable for one year when stored as directed.
Buffer AW2*	10 mL	25 mL	RT	
DEPC Water	10 mL	30 mL	RT	
Mini Column	50	200	RT	
2 mL Collection Tube	50	200	RT	

***** Prior to use, add absolute ethanol to **Buffer AW2** according to the bottle label.

Product Description

EasySC Universal RNA Purification Kit is designed for rapid purification of genomic RNA from a variety of sample sources including animal tissues and cells, plant and fungal tissues, bacteria, and blood. The specially formulated lysis buffer is used to directly lyse cells and selectively precipitate protein and DNA. The RNA supernatant is transferred to spin column, and further washed to remove impurities. Then, the RNA is eluted with DEPC water. RNA purified using this kit is ready for most downstream applications such as RT-PCR, RT-qPCR, Northern blotting, next-generation sequencing, poly A+ RNA (mRNA) purification, nuclease protection, and in vitro translation.

Features

- ❖ Fast – RNA purification process in less than 30 min.
- ❖ Versatile – Method is compatible with a variety of samples.
- ❖ High-quality – RNA is suitable for a variety of downstream applications.

Things to do before starting

- Buffer AL may form precipitates upon storage. If necessary, warm to 50°C to redissolve.
- Buffer Buffer AW2 is supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96-100%) as indicated on the bottle to obtain a working solution.
- Prepare Lysozyme working solution: dissolve lysozyme at 10 mg/mL in TE Buffer, pH 8.0. (For bacteria lysis only)
- Prepare 1X RBC Lysis Buffer. (For blood lysis only)

Protocol

1. Sample homogenization.

    - **Animal tissue:** Weigh ~20 mg tissue, and cut the tissue into small pieces, then transfer it into a 2 mL microcentrifuge tube. Add 1 mL Buffer RL, and disrupt tissue until the sample is uniform using homogenizer.
    - **Plant and fungal tissue:** Grind the plant or fungal tissue to a fine powder under liquid nitrogen using a mortar and pestle. Transfer ~100 mg power into a 2 mL microcentrifuge tube. Add 1 mL Buffer RL, and vortex thoroughly for 30 seconds.
    - **For cells grown in a monolayer:** Aspirate and discard the cell culture medium. Add 1 mL Buffer RL, and pipette up and down 3-5 times to completely lyse cells, and then transfer it into a 2 mL microcentrifuge tube.
    - **For cells grown in suspension:** Centrifuge at 500 x g for 5 minutes (<5 x 10^6 cells). Aspirate and discard the supernatant. Add 1 mL Buffer RL, and pipette up and down 3-5 times to completely lyse cells.
    - **Bacteria:** Centrifuge to collect bacteria (<1 x 10^8 cells), add 100 µL Lysozyme working solution, and incubate at RT for 10 min. Then add 1 mL Buffer RL, and vortex thoroughly for 1 min.
    - **Blood:** Transfer 500 µL fresh or frozen anticoagulated whole blood to a 2 mL microcentrifuge tube, add 1.5 mL 1X RBC Lysis Buffer, mix by inverting 5 times. Centrifuge at 2,000 x g for 5 minutes. Aspirate and discard the supernatant. Add 1 mL Buffer RL, and pipette up and down 3-5 times to completely lyse cells.

2. Incubate at RT for 5-10 min.

3. Add 400 µL ddH_2O. Shake vigorously by hand for 15 seconds, and incubate at RT for 5-15 min.

    Note: The shaking need be fast and vigorous. Slow mixing will result in insufficient extraction. Vortexing should be avoided, as it will result in DNA contamination. The addition of ddH_2O must be in proportion. Too much ddH_2O will return DNA and protein to the aqueous phase, resulting in a decrease in RNA purity.

4. Centrifuge at 12,000 x g for 15 min at RT.

    Note: After centrifugation, DNA, protein and polysaccharide form precipitate at the bottom, while RNA remains in the supernatant.

5. Transfer the supernatant to a new 2 mL microcentrifuge tube, add 0.5 volume 100% ethanol, and vortex for 10 seconds.

    Note: To obtain small RNA, add 1.5 volume 100% ethanol to the supernatant.

6. Connect a RNA Mini Column to a 2 mL Collection Tube. Transfer 600 µL sample from step 5 into the RNA Mini column. Centrifuge at 12,000 x g for 1 min.

7. Discard the filtrate and reuse the collection tube. Transfer remaining sample from step 5 to the RNA Mini Column. Centrifuge at 12,000 x g for 1 min.

8. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 12,000 x g for 1 min.

    Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.

9. Discard the filtrate and reuse the collection tube. Centrifuge the empty RNA Mini Column at 12,000 x g for 3 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.

10. Transfer the RNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 30-100 µL DEPC water to the center of membrane. Close the lid and incubate at RT for 3 min, then centrifuge at 12,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.

11. Discard the column and store the RNA at -80ºC.

Troubleshooting

Problem	Possible cause and suggestions
Low yield or decomposed	• Incomplete lysis: Sample need be immediately mixed and homogenized with Buffer RL to prevent RNA degrading. • Poor elution: Add enough DEPC water to the center of membrane, and incubate at RT for 3 min. Repeat for a second elution. • Sample storage: Repeated freeze-thaw will cause RNA degrading, avoid freeze-thaw no more than twice. • DEPC water contaminated: Use new DEPC water. • Electrophoresis: RNA may be degraded during electrophoresis. Change with new loading buffer and running buffer.
DNA contamination	• Too much sample: Reduce sample amount. • Sample rich with DNA: After lysed with Buffer RL, add 5 µL acetic acid, then add ddH_2O to extract RNA. • After addition of ddH_2O, the mixture is not shaken vigorously by band, or mixed by vortexing. • Add too much ddH_2O: The addition of ddH_2O must be in proportion with Buffer RL.
Low purity (OD260/OD280<1.65)	• Too much sample: Reduce sample amount. • Incomplete lysis: After homogenization, incubate at RT for 5-10 min. • Add too much ddH_2O: The addition of ddH_2O must be in proportion with Buffer RL. • After addition of ddH_2O, the mixture is not shaken vigorously by band, or mixed by vortexing.