Micro Sample Total RNA Rapid Extraction Kit

Cat. No.: M1521407 | Pack size: 50T | Storage: Room temperature; store at -20℃

Overview

This product is a dedicated kit for ultra-micro RNA extraction, suitable for extracting total RNA from ultra-micro amounts of cells, tissues, insects, plants, fungi, bacteria, and other samples. The typical processing range is 10^3-10^6 cells or <3 mg of tissue. This kit is safe, efficient, rapid, and convenient. It includes a proprietary DNA removal column and RNA lysis/binding reagents, yielding RNA free of DNA contamination. The optimized Trace RNA Enhancer improves RNA extraction quality. The extracted RNA can be directly used in various molecular biology experiments, including Northern blot, dot blot, mRNA purification, in vitro translation, RNase protection assay, RT-PCR, real-time RT-PCR, and cDNA library construction.

Contents & Storage

Cat. No.	Component	Size	Storage
M1521407A	Buffer MCL	33 mL	RT.
M1521407B	Buffer RB	20 mL	RT.
M1521407C	Buffer RW	38 mL	RT.
M1521407D	Buffer RW2	12 mL	RT.
M1521407E	RNase-Free ddH_2O	15 mL	RT.
M1521407F	Proteinase K	0.7 mL	RT.
M1521407G	Genomic DNA Removal Column DE (with 2 mL Collection Tube)	1 EA×50	RT.
M1521407H	Spin Column RM (with 2 mL Collection Tube)	1 EA×50	RT.
M1521407I	RNase-Free Centrifuge Tube (1.5 mL)	1 EA×50	RT.
M1521407J	Trace RNA Enhancer	280 µL	-20℃.

M1521407A, M1521407B, M1521407C, M1521407D, M1521407E, M1521407F, M1521407G, M1521407H, M1521407I: Store at room temperature for 15 months.

M1521407J: Can be stored at 4℃ for 1 month; long-term store at -20℃.

If precipitate forms in Buffer MCL, warm in a 37℃ water bath for 5-10 minutes before use to dissolve the precipitate; this does not affect performance.

Precautions

- Operators should wear disposable masks and gloves, change gloves frequently during the experiment, and use RNase-free plasticware and tips to avoid RNase contamination.
- This kit removes most DNA contamination, allowing RNA to be used directly in downstream experiments without DNase I digestion. However, nucleic acid content varies significantly among different samples or different input amounts. If downstream experiments require very high RNA purity, optional DNase I digestion can be performed.
- Fresh samples are recommended for RNA extraction. If extraction cannot be performed immediately, samples should be frozen in liquid nitrogen and stored below -70°C. Avoid repeated freeze-thaw cycles, as this will affect RNA yield and quality.
- For micro samples such as nematodes, mites, and pollen, homogenization tube C must be purchased separately.

Protocol

Preparation before use: Add 48 mL of absolute ethanol to 12 mL of Buffer RW2.

1. Homogenization

Add ground plant, animal, fungal, or other samples (bacterial and cell samples do not require grinding) to a 1.5 mL RNase-Free centrifuge tube pre-filled with 600 µL Buffer MCL, 10 µL Proteinase K, and 5 µL Trace RNA Enhancer. Immediately vortex vigorously for 30 seconds to lyse thoroughly. Centrifuge at 14,000 rpm (20,000 × g) for 2 minutes.

Note: For micro samples such as nematodes, mites, and pollen, add 600 µL Buffer MCL and 10 µL Proteinase K to homogenization tube C, then add the tissue sample. Grind using a tissue grinder for 30-60 seconds, twice (adjust the machine frequency to avoid breaking the tube). Add 5 µL Trace RNA Enhancer, immediately vortex vigorously for 30 seconds to mix and lyse thoroughly, then centrifuge at 14,000 rpm (18,630 × g) for 2 minutes.

2. Transfer all supernatant to a Genomic DNA Removal Column DE (placed in a collection tube). Centrifuge at 14,000 rpm (18,630 × g) for 30 seconds. Discard the removal column and keep the flow-through.

3. Add 0.7 volumes of Buffer RB relative to the flow-through volume (precipitation or flocculent material may appear - this is normal). Mix well, then transfer the mixture to a Spin Column RM (placed in a collection tube). Centrifuge at 14,000 rpm (18,630 × g) for 2 minutes. Discard the flow-through and return the column to the collection tube.

4. Add 700 µL Buffer RW to the column. Centrifuge at 12,000 rpm (13,680 × g) for 30 seconds. Discard the flow-through and return the column to the collection tube.

5. Add 500 µL Buffer RW2 (ensure absolute ethanol has been added before use) to the column. Centrifuge at 12,000 rpm (13,680 × g) for 30 seconds. Discard the flow-through and return the column to the collection tube.

6. Repeat step 5.

7. Centrifuge at 12,000 rpm (13,680 × g) for 2 minutes.

8. Transfer the column to a new 1.5 mL RNase-Free centrifuge tube. Open the lid and let stand at room temperature for 1 minute to completely air-dry any residual ethanol. Add 10-15 µL RNase-Free ddH_2O to the center of the membrane without touching it. Let stand at room temperature for 1 minute, then centrifuge at 12,000 rpm (13,680 × g) for 30 seconds. The obtained RNA can be used directly in downstream experiments or stored below -80°C.

Note: The volume of RNase-Free ddH_2O should not be less than 10 µL; smaller volumes will reduce recovery. To increase RNA yield, reload the first eluate onto the column for a second elution, or preheat the RNase-Free ddH_2O to 65°C.

RNA Integrity, Concentration, and Purity Assessment

Integrity

RNA integrity can be assessed by standard agarose gel electrophoresis (conditions: 1-1.2% gel, 0.5× TBE running buffer, 120-150 V for 15-25 minutes). Since rRNA accounts for more than 80% of total RNA, very distinct rRNA bands should be visible under UV after electrophoresis. The types and sizes of rRNA vary among species:

- Animals: 28S (4.7-5.0 kb), 18S (1.9 kb), 5S (120 bp)
- Plants: 25S (3.7 kb), 18S (1.9 kb), 5S (120 bp)
- E. coli: 23S (2.9 kb), 16S (1.5 kb), 5S (120 bp)
- Insects/Mollusks: The 18S band is typically brighter than the 28S band.

If the bands are smeared and diffuse, it generally indicates severe RNA degradation. Re-run the gel or re-extract the RNA. Otherwise, qPCR may still yield Ct values, but accuracy and reproducibility will be poor.

Concentration

Determine RNA concentration by measuring absorbance at 260 nm (A_{260}) using a spectrophotometer. Use RNase-Free ddH_2O for blanking. One absorbance unit at 260 nm corresponds to 40 µg/mL RNA.

Purity

The A_{260}/A_{280} ratio is used to assess nucleic acid purity. A ratio between 1.8 and 2.1 indicates high-purity RNA. The A_{260}/A_{280} ratio is dependent on pH and ionic strength; as pH increases, A_{280} decreases while A_{260} remains unchanged, leading to an increased ratio. Because water is typically acidic, it can lower the A_{260}/A_{280} ratio. We recommend using a weakly alkaline buffer (e.g., TE, pH 8.0) as the diluent and blank control to obtain accurate, reproducible readings.

Storage and Shipping

Attribute	Value
Storage Conditions	Room temperature, Store at -20°C
Shipped In	Ice chest + Ice pads

Attribute	Value
Stability And Storage	Each component has a shelf life of 15 months under corresponding storage conditions.

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