Magnetic Tissue RNA Plus Kit

Cat.No.: HYC326

Product Description

This kit delivers a simple and high-performance protocol for total RNA extraction from animal tissues. After physical disruption of tissue samples, RNA in the lysate binds to silica-coated magnetic beads under high-salt conditions. Impurities such as proteins are washed away with wash buffers, and genomic DNA is eliminated via DNase I digestion. Purified RNA is then washed and eluted into RNase-free water.

No toxic hazardous reagents including β-mercaptoethanol, phenol or chloroform are required throughout the workflow, yielding high-integrity RNA compatible with diverse downstream applications such as RT-PCR, Real-Time PCR, microarray analysis and in vitro translation.

Components

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DNase I (RNase Free)	4×1000 U
10×Reaction Buffer	2×1000 μL
Buffer PL1	50 mL
Buffer PL2	15 mL
Buffer RW1	50 mL
Buffer RW2*	33 mL
RNase-Free Water	8 mL
Magbeads PN	5 mL

Storage

Store Buffer PL1, DNase I (RNase Free) and 10× Reaction Buffer at 2–8 °C. All other components should be kept at room temperature (10–30 °C).

Required Reagents

Absolute Ethanol, Isopropanol

Instructions

Preparations

1. Use RNase-free plastic consumables and pipette tips to prevent nuclease contamination.
2. Dry bake glassware at 180 °C for 4 hours before use. Soak plasticware in 0.5 M NaOH for 10 min, rinse thoroughly with pure water, then autoclave.
3. Wear disposable masks and gloves during the experiment; replace gloves frequently.
4. Add anhydrous ethanol to Buffer RW2 and mix thoroughly as instructed on the bottle label prior to first use.
5. Recommended input amount of tissue samples ranges from 15–25 mg. For liver, spleen, kidney and other organs, the loading amount should not exceed 20 mg, otherwise gDNA residue or reduced RNA yield may occur.
6. Avoid repeated freeze-thaw cycles of tissue samples, which will impair RNA yield and integrity. If extraction cannot be performed immediately, snap-freeze fresh tissues in liquid nitrogen and store at −85 ~ −65 °C; repeated freeze-thaw should be avoided.
7. Mix thawed tissues with Buffer PL1 promptly after taking out from low-temperature storage. Do not wait until tissues are fully thawed to prevent RNA degradation.
8. Thoroughly grind or homogenize tissues; keep samples cool during homogenization to avoid RNA degradation induced by excessive heat.
9. Instructions for DNase I (RNase Free): Add 500 µL RNase-free water to the cryovial containing DNase I (RNase Free). Mix by gentle inversion; do not vortex. The reconstituted DNase I stock concentration is 2 U/µL, store at −20 ± 5 °C.

Operating Protocol

1. Add 500 µL Buffer PL1 to ground tissue samples, vortex for 5 sec for full resuspension (avoid excessive vortexing). Incubate for 5 min, then centrifuge at 12,000 rpm for 4 min at 4 °C. Transfer all supernatant into a new 1.5 mL centrifuge tube.
2. Add 120 µL Buffer PL2, 300 µL isopropanol and 50 µL Magbeads PN to the tube, then vortex for 5 sec to fully resuspend. Incubate the tube on a thermomixer with shaking at 1,000 rpm and 25 °C for 6 min, or mix by continuous inversion for 6 min.
   Note: Keep Magbeads PN suspended throughout incubation.

3. Place the tube on a magnetic rack and stand for 1 min. Flip the magnetic rack to fully capture Magbeads PN onto the tube wall while keeping the tube secured on the rack. Open the lid and carefully aspirate all liquid without touching the captured magnetic beads.
4. Add 500 µL Buffer RW1 to the tube and vortex for 5 sec to resuspend Magbeads PN. Shake on a thermomixer at 1,200 rpm and 25 °C for 1 min, or mix by continuous inversion for 1 min.

   Note: Maintain Magbeads PN in suspension during shaking.
5. Set the tube on a magnetic rack and incubate for 1 min. Flip the rack to fully adsorb Magbeads PN to the tube wall with the tube fixed on the rack. Open the lid and gently remove all supernatant without disturbing the beads.
6. Add 80 µL DNase I working solution to the tube, vortex for 5 sec to resuspend Magbeads PN, then incubate at 25 ± 5 °C for 8 min.
7. Preparation of DNase I working solution: Mix 52 µL RNase-free water, 8 µL 10× Reaction Buffer and 20 µL DNase I stock (2 U/µL).
8. Add 700 µL Buffer RW2 to the tube and vortex for 5 sec to resuspend Magbeads PN. Shake on a thermomixer at 1,200 rpm and 25 °C for 1 mi n, or mix by repeated inversion for 1 min.

   Notes:

   1) Ensure Magbeads PN remain suspended during shaking.

   2) Confirm anhydrous ethanol has been added to Buffer RW2 as labeled before use.
9. Place the tube on a magnetic rack for 1 min. Flip the rack to fully capture beads on the tube wall while the tube stays fixed on the rack. Open the lid and carefully aspirate all liquid without touching the magnetic beads.
10. Repeat Steps 8 and 9 once.
11. Leave the tube lid open and air-dry at ambient temperature for 3 min.

    Note: This step eliminates residual ethanol to prevent inhibition of downstream enzymatic reactions (digestion, PCR, etc.).
12. Add 50–70 µL RNase-free water to the tube and vortex for 5 sec to resuspend Magbeads PN. Incubate on a thermomixer with shaking at 1,000 rpm and 65 °C for 6 min, or mix by continuous inversion for 6 min.

    Note: Keep Magbeads PN suspended during incubation.

13. Place the tube on a magnetic rack and stand for 1 min until all Magbeads PN are captured on the tube wall. Carefully transfer the supernatant containing RNA to a new user-supplied centrifuge tube, and store at −70 ± 10 °C.
14. (Optional) Supplement eluted RNA with RNase Inhibitor to a final concentration of 1 U/μL to effectively mitigate RNA degradation.
    Note: RNase Inhibitor is not included with this kit by default.