SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318 E-mail: sales@sunlongbiotech.com
www.sunlongbiotech.com

Total RNA Extraction Kit

Cat No.: EXR0222

Package: 50T/100T

Kit Contents:

Components	50T	100T	Storage	Validity Period
Lysis Buffer	50ml	100ml	2-8°C	1 year
Washing Buffer	15ml	15ml×2	RT	1 year
Balance Buffer	50ml	50ml×2	RT	1 year
RNase free ddH_2O	15ml	15ml×2	RT	1 year
RNase free Adsorption Column	50	100	RT	1 year
RNase free Collection Tubes (2ml)	50	100	RT	1 year

Reagents Required but Not Provided

Absolute ethanol, add 60ml to 15ml washing buffer before use.

Protocol

1. Homogenizing samples.

a. Plant sample: Place fresh or -70°C freezing 100mg samples in liquid nitrogen and grind thoroughly with a mortar and pestle, transfer the sample powder into 1mL lysis buffer.

b. Animal Tissue: Add 1ml lysis buffer to fresh or -70°C freezing 100mg samples and grind using a mortar and pestle or homogenize using a homogenizer.

c. Adherent Cell: Add 1mL lysis buffer per 10^6 cells in the culture dish. Pipette the lysis buffer up and down several times.

d. Suspension Cell: Harvest cells by centrifugation. Add 1 ml of lysis buffer per 10^6 cells from animal, plant or yeast, or 1×10^7 cells of bacterial.

e. Blood: Take 0.2-1mL fresh blood, and add three times volumes of Red Blood Cell Lysis Buffer (R1010). Mix thoroughly. Incubate for 10 minutes at room temperature. Centrifuge the sample at 10000rpm for 1min and discard supernatant. If precipitation contains red cells, add 2 times volume of Red Blood Cell Lysis Buffer (R1010) and lyse again and repeat above steps. Add 1 ml lysis buffer to precipitated mixture after centrifugation and mix thoroughly.

2. Incubate homogenized samples at room temperature for 5 min to permit complete dissociation of the nucleoprotein complex.

3. Add 200 µl of chloroform to homogenized samples. Cap the tube securely and vortex for 15 s. Incubate for 3-5 minutes at room temperature.

4. Centrifuge the sample for 10 min at 12,000 rpm at 2-8°C. RNA remains mainly in the aqueous phase. Pipette the aqueous phase out into a new tube. Be careful not to absorb the precipitation.

5. Preparation of RNase-free adsorption column: Add 500μL balance buffer in adsorption column, incubate for 2 minutes at room temperature. Centrifuge for 2 min at 12,000 rpm at 2-8°C. Discard the flow-through.
6. Add 200μL ethanol in the aqueous phase from step 4 and votex, tansfer the aqueous phase in adsorption column, and stay for 2 min. Centrifuge for 2 min at 12,000 rpm at 2-8°C and discard the flow-through.
7. Add 600μL washing buffer **(Ensure that ethanol is added before use)** in adsorption column, Centrifuge for 2 min at 12,000 rpm at 2-8°C and discard the flow-through.
8. Add 600μL washing buffer in adsorption column, centrifuge for 2 min at 12,000 rpm at 2-8°C and discard the flow-through.
9. Centrifuge for 2 min at 12,000 rpm, open the cap and incubate for a few minutes to dry the adsorption column membrane, ensure that no ethanol is carried over during RNA elution.
10. Place the adsorption column in a clean RNase-free centrifuge tube. Add 50-100 μl RNase-free water directly to the adsorption column membrane. Incubate for 5 min at room temperature and centrifuge for 2 min at 12,000 rpm (~13,400× g) to elute the RNA.

Notes

1. All related vessels and consumables should be RNase-free products, operating process carefully. Wear gloves and gauze mask when handling RNA and all reagents, as skin is a common source of RNase.
2. OD value of RNA in the aqueous solution may be between 1.5and 1.9, that doesn't mean RNA contaminated, need electrophoresis ensure.