VeZol-Pure Total RNA Isolation Kit

RC202

Version 24.1

Product Description

VeZol-Pure Total RNA Isolation Kit is applicable for isolating high-quality Total RNA from various samples such as cultured cells, animal and plant tissues. This kit combines phenol/guanidine-based lysis of samples with silica membrane-based purification of total RNA, with the advantages of strong lysing ability, wide compatibility, fast, high yields, and good purity. Samples are fully lysed with VeZol Reagent, and RNA is specifically adsorbed from the aqueous phase by silica membrane. The obtained total RNA can be directly used for molecular biology experiments such as RT-PCR, qRT-PCR, Northern Blot, Dot Blot, in vitro translation, and high-throughput sequencing.

Components

	Components	RC202-01 (100 rxns)
BOX 1	VeZol Reagent	100 ml
	Buffer RWA	32 ml
	Buffer RWB	22 ml
BOX 2	RNase-free ddH_2O	30 ml
	FastPure RNA Columns VI (each in a 2 ml Collection Tube)	100
	RNase-free Collection Tubes 1.5 ml	100

Storage

Store BOX 1 at 2 ~ 8°C and protect from light. Transport at room temperature.

Store BOX 2 at 15 ~ 25°C and transport at room temperature.

Applications

It is applicable for a variety of cultured cells, animal tissues, and plant tissues.

Self-prepared Materials

Chloroform (substitute: 1-Bromo-3-chloropropane), absolute ethanol, RNase-free centrifuge tube.

Notes

For research use only. Not for use in diagnostic procedures.

1. Please add corresponding volume of absolute ethanol to Buffer RWA and Buffer RWB according to the label, mark the bottle body and cap and mix well before use.
2. This product contains phenol, which is toxic and corrosive. Protective equipment such as protective clothing, gloves, goggles, and masks should be worn when using it. If it accidentally comes into contact with the eyes, rinse immediately with plenty of H_2O and seek medical treatment. If it comes into contact with the skin, immediately rinse with plenty of detergent and H_2O. If there is still discomfort, please seek medical treatment.
3. The key to RNA extraction is preventing RNase contamination. RNase, which is prevalent in the environment and extremely stable, can rapidly degrade RNA, even in trace amounts. Therefore, take all necessary precautions as per the conventional RNA extraction procedure, including wearing a mask and sterile disposable gloves, working in a separate clean area, and using RNase-free labware.

Experiment Process

1. Sample Processing

◇ **Animal and plant tissues**

a. Transfer frozen or fresh tissues to a mortar precooled with liquid nitrogen. Grind with a pestle while adding liquid nitrogen until the sample is ground into powder (with no obvious granules). Add 1 ml of VeZol Reagent per 50 - 100 mg of tissue, and vortex until there are no obvious clumps. Incubate at room temperature for 5 min.

▲ Insufficient grinding will affect the yield and quality of RNA.

▲ When the plant tissue input is ≤50 mg, extraction process can be performed without chloroform: add 600 µl of VeZol Reagent, mix by vortexing, incubate at room temperature for 5 min, and centrifuge at 12,000 rpm (13,400 × g) at 4°C for 5 min. Then take 500 µl of the supernatant and add 0.5 times the volume of absolute ethanol, mix thoroughly, and then proceed to total RNA extraction/step e.

◇ **Adherent cells**

a. Discard the culture medium and rinse once with 1 × PBS.

b. Add 1 ml of VeZol Reagent per well of a regular 6-well cell culture plate or per 3.5 cm diameter dish (approximately 10 cm^2 culture area). Allow the VeZol Reagent to fully cover the cell layer, and then detach the cells by pipetting repeatedly.

c. Transfer the mixture to a RNase-free centrifuge tube, pipette up and down until the cells are completely lysed, and incubate at room temperature for 5 min.

◇ **Suspended cells**

a. Centrifuge at 2,300 rpm (500 × g) and 4°C for 2 - 5 min and discard the supernatant to collect the cells.

b. Add 1 ml of VeZol Reagent per 5 × 10^6 - 1 × 10^7 cells.

c. Vortex or pipette up and down until the cells are completely lysed, and incubate at room temperature for 5 min.

▲ Frozen cells should be vortexed immediately after the addition of VeZol Reagent to avoid incomplete lysis.

◇ **Blood sample**

a. Take fresh blood, add 900 µl VeZol Reagent per 100 µl of blood.

b. Vortex or pipette up and down until the cells are completely lysed, and incubate at room temperature for 5 min.

2. Total RNA Extraction

a. Add 1/5 volume of chloroform to the above lysate. Shake vigorously for 15 sec to obtain an emulsion, and incubate at room temperature for 5 min.

b. Centrifuge at 12,000 rpm (13,400 × g) and 4°C for 5 min.

c. Carefully take out the centrifuge tube. The mixture separates into three layers: an upper aqueous phase (containing RNA), an interphase, and a lower red organic phase. Carefully transfer the upper aqueous phase (about 500 µl) to a new RNase-free centrifuge tube. Do not disturb the interphase.

d. Slowly add an amount of absolute ethanol equal to 0.5 times the volume of the aqueous phase, mix thoroughly.

▲ After adding ethanol, the solution becomes turbid or forms a flocculent precipitate, which is a normal phenomenon. It can be directly proceeded to the next step by mixing with vortexing.

e. Transfer the mixture from the previous step (including precipitate) to FastPure RNA Columns VI (RNA Columns have been placed in collection tubes), centrifuge at 12,000 rpm (13,400 × g) at 4°C for 30 sec, discard the filtrate.

f. Add 700 µl Buffer RWA (already added absolute ethanol) to FastPure RNA Columns VI, centrifuge at 12,000 rpm (13,400 × g) at 4°C for 30 sec, discard the filtrate.

g. Add 500 µl Buffer RWB (already added absolute ethanol) to FastPure RNA Columns VI, centrifuge at 12,000 rpm (13,400 × g) at 4°C for 30 sec, discard the filtrate.

h. Repeat step g once.

i. Put the FastPure RNA Columns VI back into the collection tube, and centrifuge at 12,000 rpm (13,400 × g) at 4°C for 2 min to prevent ethanol contamination.

j. Be careful to transfer the spin column to a new RNase-free Collection Tube 1.5 ml centrifuge tube, and add 50 - 200 µl of RNase-free ddH_2O to the center of the spin column. Let it stand at room temperature for 1 min, centrifuge at 12,000 rpm (13,400 × g) at 4°C for 1 min, and elute the RNA.

▲ To increase yield, preheat RNase-free ddH_2O at 65°C, or extend room temperature incubation time to 5 min, or perform a second elution.

k. The extracted Total RNA can be directly used for downstream experiments or stored at -85 ~ -65°C.