# YTA-Total RNA Extraction mini Kit plus YTzol

Cat No: YT9001 Size: 50 preps <<for research use only>>

Kit Contents	50 preps
YTzol Lysis buffer	50 ml
Wash buffer 1	30 ml
Wash buffer 2 (concentrate)	20 ml
RNase free water	10 ml
Mini column	50 each
Elution tube	50 each
Collection tube	50 each

Preparation note:
Add 80ml ethanol (96-100%) to wash 2

Materials be supplied by the users :

Ethanol (96–100%)

## **Description**

YTA- Total RNA Extraction Kit plus YTzol for Tissue offers a rapid and reliable method for isolating total RNA from a wide variety of sample types and input amounts.. Samples are first lysed and homogenized in the presence of guanidinium thiocyanate, a chaotropic agent that effectively inactivates endogenous RNases and protects RNA integrity. Following homogenization, ethanol is added to promote RNA binding to the silica-based spin column membrane. Contaminants and impurities are efficiently removed through sequential wash steps, and the purified total RNA is finally eluted in RNase-Free Water, ready for use in a range of downstream applications, including RT-PCR, qPCR, and RNA sequencing.

### **Features**

- Consistent and stable RNA yield
- High-purity total RNA suitable for sensitive applications
- No chloroform extraction, phase separation, or precipitation required
- Fast and efficient RNA extraction using YTzol reagent

#### **Notes**

- Use only sterile, disposable, individually wrapped plasticware.
- Employ RNase-free pipette tips and microcentrifuge tubes at all stages.
- Always wear disposable gloves to prevent RNase contamination from skin contact; change gloves frequently, especially when handling purified RNA.
- Follow aseptic and RNase-free techniques throughout the procedure.
- Perform all extraction steps on ice to preserve RNA stability.
- Use the recommended volume of YTzol reagent according to sample type and quantity.

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10cm <sup>2</sup> adherent cells	1 ml
10 <sup>7</sup> suspension cells	1-2 ml
100 ul white cells	2 ml
50-100 mg ordinary tissue	1 ml
50-100 mg special tissue(live,spleen,bone or cartilage)	2ml
15-100 mg plant tissue	1 ml

## **Protocol**

1. Sample process

<u>Tissues from animal or plant</u>(either fresh or frozen at -70 °C until use) can be processed by freezing with liquid nitrogen and grinding into a fine powder using a mortar and pestle. Homogenize tissue samples in 500μL to 1 ml YTzol per 10-50 mg tissue using a tissue homogenizer or rotor-stator.

<u>Adherent Cells</u>: Lyse cells directly in a culture dish by adding 1 ml of YTzol to the dish and passing the cell lysate several times through a pipet tip. The amount of YTzol required is based on the culture dish area (1 ml per 10 cm2) and not on the number of cells present.

<u>Suspension Cells</u>: Harvest cells and pellet cells by centrifugation. Use 1 ml of the YTzol per  $5-10 \times 10^6$  animal, plant, or yeast cells, or per  $1 \times 10^7$  bacterial cells. Lyse cells by repetitive pipetting up and down. Do not wash cells before addition of YTzol to avoid any mRNA degradation.

Disruption of some yeast and bacterial cells may require a homogenizer.

- 2. Incubate at 15-30°C for 5 min, to lyse the nucleoprotein complex completely.
- 3. Centrifuge or spin to remove particulate debris from homogenized tissue and transfer the supernatant into a new nuclease free tube.
- 4. Add an equal volume of ethanol. Mix well. Avisible may form after adding ethanol.
- 5. Place a Mini Column to a Collection Tube and transfer the ethanol added sample mixture to the Mini Column. Centrifuge at full speed for 1 min, discard the flow-through and return the Mini Column back to the Collection Tube.
- 6. Add 500 µl of Wash Buffer 1 to the Mini Column, centrifuge at at full speed for 1 min. Discard the flow-through and return the Mini Column back to the Collection Tube.
- 7. Add 750 µl of Wash Buffer 2 to the Mini Column, centrifuge at full speed for 1 min. Discard the flow-through and return the Mini Column back to to the Collection Tube. -- Note: Make sure that ethanol has been added into Wash Buffer 2 when first use.
- 8. Repeat step 7 for one more washing.
- 9. Centrifuge the Mini Column at full speed for an additional 3 min to dry the Mini Column --Important Step! This step will avoid the residual- liquid to inhibit subsequent enzymatic reaction.
- 10. Place the Mini Column to a Elution Tube (provided, 1.5 ml microcentrifuge tube).
- 11. Add 20-50 µl of RNase-free ddH2O to the membrane center of the Mini Column. Stand the Mini Column for 1 min. -- Important Step! For effective elution, make sure that RNase-free ddH2O is dispensed on the membrane center and is absorbed completely. -- Important: Do not elute the RNA using RNase-free water less than suggested volume (< 40 µl). It will lower the RNA yield.
- 12. Centrifuge the Mini Column at full speed for 1 min to elute RNA.
- 13. Store RNA at -70C.

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