

# TransZol Up Plus RNA Kit

Please read the datasheet carefully prior to use

Cat. No. ER501

**Storage:** *TransZol Up* at 2-8°C away from light for one year, others at room temperature (15°C- 25°C) in a dry place for one year

## Description

*TransZol Up Plus RNA Kit* is suitable for the isolation of total RNA from cells and tissues. After the sample is lysed with *TransZol Up* and RNA Extraction Agent is added, the solution separates into an upper colorless aqueous phase (containing RNA), an interphase and a lower pink organic phase. A silica-based spin column is used to specifically bind to RNA in the aqueous phase. Compared with other total RNA extraction methods, *TransZol Up Plus RNA Kit* possesses not only advantages of strong lysis capability, high yield and a wide range of applications of *TransZol Up*, but also the advantage of high purity of spin column extraction.

## Highlights

- **High operational safety:** RNA Extraction Agent replaces chloroform.
- A wide range of applications enabled by the maximum binding capacity of the spin column of 100 µg: animal and plant tissues, viruses, bacteria or other samples. Small amount of samples (50-100 mg tissues,  $5 \times 10^6$  cells, or 200 µl blood) and large amount of samples ( $\geq 1$ g tissues or  $\geq 10^7$  cells).
- Strong lysis capability: Complete and fast lysis, high yield.
- Rapid extraction: Completed in one hour.
- Visible workflow: Pink organic phase to facilitate separation from colorless aqueous phase
- High purity: Minimum DNA and protein contamination.

## Kit Contents

Component	ER501-01 (100 rxns)
TransZol Up	100 ml
RNA Extraction Agent	20 ml
Clean Buffer 9 (CB9)	110 ml
Wash Buffer 9 (WB9)	24 ml
RNase-free Water	40 ml
RNase-free Tube (1.5 ml)	100
RNA Spin Columns with Collection Tubes	100

## Procedures

Set the temperature of the low-temperature centrifuge at 2-8°C in advance. Add 96 ml of absolute ethanol to WB9 before use.

Reagents provided by customers: absolute ethanol

### 1. Homogenization

#### a. Adherent cells

- Discard the culture medium, and wash the culture dish once with 1×PBS
- Add 1ml of *TransZol Up* per 10 cm<sup>2</sup> of growth area of cells, incubate horizontally for a while to make the lysis buffer evenly cover the surface of cells and disrupt cells. Detach cells by pipetting (for strongly adherent cells, detach cells with a cell scraper).
- Transfer the lysate to a microcentrifuge tube. Add 0.2 ml RNA Extraction Agent Pipetting up and down until the lysate contains no visible precipitate.
- Incubate at room temperature for 5 minutes.

#### b. Bacteria and suspension cells

- Transfer the bacteria or suspension cells with the culture medium together to a microcentrifuge tube. Centrifuge the tube at 8,000×g for 2 minutes at 2-8°C, and discard the supernatant.
- Add 1 ml of *TransZol Up* per  $\leq 2 \times 10^9$  bacteria or per  $\leq 10^7$  cells.
- Add 0.2 ml RNA Extraction Agent. Pipetting up and down until no visible precipitate is present in the lysate.
- Incubate at room temperature for 5 minutes.

#### c. Blood samples

- Add 1ml of *TransZol Up* per  $\leq 200$  µl blood. It is recommended that the blood volume should not be less than 50 µl.



- Add 0.2 ml RNA Extraction Agent. Pipette up and down thoroughly to mix well.
  - Incubate at room temperature for 5 minutes.
- d. Animal and plant tissues
- After weighing samples frozen at ultra-low temperature, quickly transfer into a precooled mortar with liquid nitrogen. Grind thoroughly into powder. Use more liquid nitrogen if needed. Incomplete grind can affect RNA yield and quality.
  - Transfer the tissue powder to a microcentrifuge tube. Add 1 ml of *TransZol Up* and 0.2 ml of RNA Extraction Agent per 50-100 mg tissue. Homogenize tissue samples with a homogenizer or pipette up and down to mix well.
  - Incubate at room temperature for 5 minutes.
2. Centrifuge the tube at 10,000×g for 15 minutes at 2-8°C . The mixture separates into a lower pink organic phase, an interphase, and a colorless upper aqueous phase which contains RNA. The volume of the aqueous phase is about 50%- 60% volume of the *TransZol Up* reagent used.
  3. Transfer the colorless aqueous phase containing RNA to a new microcentrifuge tube (to avoid DNA contamination from the interphase, a certain portion of aqueous phase can be left). Add an equal volume of absolute ethanol (precipitates may appear at this stage). Mix gently by inverting the tube.  
**All the following centrifugation steps can be carried out at room temperature.**
  4. Transfer the generated solution and precipitates together to a spin column. Centrifuge at 12,000×g for 30 seconds at room temperature. Discard the flow through (if the volume of the mixture is larger than the capacity of spin column, repeat this step until all the mixture has been processed).
  5. Add 500 µl of CB9 to the spin column. Centrifuge at 12,000×g for 30 seconds at room temperature. Discard the flow through.
  6. Repeat step 6 once.
  7. Add 500 µl of WB9 (check to make sure that absolute ethanol has been added) into the spin column. Centrifuge at 12,000×g for 30 seconds at room temperature. Discard the flow through.
  8. Repeat step 8 once.
  9. Centrifuge at 12,000×g for 2 minutes at room temperature in order to completely remove the residual ethanol.
  10. Place the spin column into an RNase free tube (supplied in the kit). Add 50-200 µl of RNase-free Water into the center of the spin column and incubate at room temperature for 1 minute.
  11. Centrifuge at 12,000×g for 1 minutes at room temperature to elute RNA.  
(Optional: to obtain more RNA, it is recommended to repeat Step 11 & Step 12 to perform the second elution)
  12. Store the isolated RNA at -80°C.

#### Notes

- It is important to vortex thoroughly after adding RNA Extraction Agent to ensure good isolation result.
- Ensure that all the organic reagents (including chloroform, absolute ethanol, etc.) and consumables (such as microcentrifuge tubes and pipette tips) used are RNase-free.
- Although the binding capacity of the column is large (up to 100 µg), the elution ability of RNase-free Water is limited. Therefore, second elution is recommended for large amount of samples.
- It is recommended to use RNA Extraction Agent for RNA extraction, and chloroform can also be used instead of RNA Extraction Agent.

FOR RESEARCH USE ONLY

