# *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 chloroform 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

- 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, $5\times10^6$ cells, or 200 μl blood) and large amount of samples (≥1g tissues or $\geq10^7$ 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

| Components | ER501-01 (100 rxns) |
|---|---|
| *TransZol* Up | 100 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 Column 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: chloroform, 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 $cm^2$ of growth area of cells, incubate horizontally for a while to make the lysis buffer evenly coverthe 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. 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 $\leq2\times10^9$ bacteria or per $\leq10^7$ cells.
- 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≤200 μl blood. It is recommended that the blood volume should not be less than 50 μl.
- 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 1ml of *TransZol* Up 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. Add 0.2 ml of chloroform or 50 µl of 4-Bromoanisole per ml *TransZol* Up. Vortex the tube vigorously for 30 seconds. Incubate at room temperature for 3 minutes.
3. 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.
4. 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.
5. 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).
6. Add 500 µl of CB9 to the spin column. Centrifuge at 12,000×g for 30 seconds at room temperature. Discard the flow through.
7. Repeat step 6 once.
8. 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.
9. Repeat step 8 once.
10. Centrifuge at 12,000×g for 2 minutes at room temperature in order to completely remove the residual ethanol.
11. 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.
12. 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)
13. Store the isolated RNA at -80°C.

Notes
- It is important to vortex thoroughly after adding chloroform 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.