MagicPure® Total RNA Kit

Please read the datasheet carefully prior to use.

Cat. No. EC521

Version No. Version 1.0

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

The kit is designed for the isolation of total RNA from cultured cells, microorganisms, and animal and plant 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. After collecting the aqueous phase, the RNA can be recovered by magnetic bead-specific adsorption. Compared with other total RNA extraction methods, it has the advantages of strong lysis ability, high yield, wide application range and so on. This kit is suitable for high-throughput automated nucleic acid extractor adopting magnetic rod technology.

Features

- High operational safety: RNA Extraction Agent replaces chloroform.
- A wide range of applications: suitable for samples such as cultured cells, animal and plant tissues, viruses and bacteria.
- Easy operation: few steps outside the instrument.
- High purity: minimum DNA and protein contamination.

Kit Contents

Component	EC521-01/11 (50 rxns)
TransZol Up	55 ml
RNA Extraction Agent	15 ml
Binding Buffer 44 (BB44)	10 ml
Magnetic Total RNA Beads	1.3 ml
Clean Buffer 44 (CB44)	85 ml
Wash Buffer 44 (WB44)	12 ml
RNase-free Water	6 ml
Magnetic Stand (16 hole)	1/-

Prepare before operation

Set the temperature of the low-temperature centrifuge at 2-8°C in advance, add 30 ml of isopropanol to BB44 before use, and add 48 ml of absolute ethanol to WB44 before use.

Reagents not included in the kit: isopropanol, absolute ethanol.

Procedures

1. Homogenization

Adherent cells

- Discard the culture medium, and wash the culture dish once with 1×PBS.
- Add 1ml of *TransZol* Up per 10 cm² of growth area of cells, incubate horizontally for a while to make the lysis buffer evenly cover the surface of cells and lyse 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. Pipet up and down until the lysate contains no visible precipitate. Vortex and shake at room temperature for 5 minutes.

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 ≤2×10^9 bacteria or per ≤5×10^6 cells.
• Add 0.2 ml RNA Extraction Agent. Pipet up and down until the lysate contains no visible precipitate. Vortex and shake at room temperature for 5 minutes.

Animal and plant samples

• After weighing sample frozen at ultra-low temperature, quickly transfer it into a precooled mortar with liquid nitrogen. Grind it thoroughly into powder. Use more liquid nitrogen if needed. Incomplete grind can affect RNA yield and quality.
• Transfer the powdered sample to a microcentrifuge tube. Add 1ml of *TransZol* Up per 50-100 mg sample.
• Add 0.2 ml of RNA Extraction Agent. Pipet up and down until the lysate contains no visible precipitate. Vortex and shake at room temperature for 5 minutes.

2. Centrifuge the tube at 10,000×g at 2-8°C for 15 minutes . 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 (to avoid DNA contamination from the interphase, a certain portion of aqueous phase can be left).
3. Transfer the colorless aqueous phase containing RNA to a new microcentrifuge tube. Add an equal volume of BB44 (check if isopropanol has been added before use) and 25 µl of Magnetic Total RNA Beads (mix by vortexing before magnetic beads use).
4. Vortex and mix for 12 minutes.
5. Place the centrifuge tube on the magnetic stand to perform magnetic separation, pipet the liquid other than the magnetic beads, and avoid pipetting the magnetic beads. (Suggestions for magnetic separation operation: after the centrifuge tube is placed on the magnetic stand, gently rotate left and right, and after the magnetic beads gather on the tube wall close to the magnetic stand, gently reverse the magnetic stand 2-3 times, so that the magnetic beads on the tube cover also gather to the tube wall, and let stand for 30 seconds.)
6. Take the centrifuge tube down, add 800 µl CB44, vortex and mix for 1 minute, then perform magnetic separation. Pipet the liquid other than the magnetic beads, and avoid pipetting the magnetic beads.
   (Try to absorb the liquid as much as possible, if there is liquid residue on the tube wall, you can briefly centrifuge and then perform magnetic separation, and each of the following steps of magnetic separation can be done)
7. Repeat step 6 once.
8. Take the centrifuge tube down, add 500 µl WB44 (check if absolute ethanol has been added before use), vortex and mix for 1 minute, then perform magnetic separation. Pipet the liquid other than the magnetic beads, and avoid pipetting the magnetic beads.
9. Repeat step 8 once.
10. Place the centrifuge tube on the magnetic stand and let dry at room temperature for 5 minutes.
11. Remove the centrifuge tube, add 50-100 µl RNase-free Water, thoroughly mix by pipetting up and down, and incubate at 56°C for 5 minutes.
12. Place the centrifuge tube on the magnetic stand for magnetic separation, and pipet the liquid other than the magnetic beads into a sterile 1.5 ml centrifuge tube, avoid pipetting the magnetic beads. Store RNA at -70°C.

Notes

• It is important to vortex thoroughly after adding RNA Extraction Agent to ensure good isolation result.
• Be sure to vortex and mix the beads before use.
• Ensure that all the organic reagents and consumables (such as microcentrifuge tubes and pipette tips) used are RNase-free.

For research use only, not for clinical diagnosis.

Version number: V1-202302
Service telephone +86-10-57815020
Service email complaints@transgen.com