User guide

TRI [Trizol] REAGENT

TRI [Trizol] reagent
Cat. #BT02AL - 100 ml
Store at 2-8˚C

Product Description
TRI Reagent is a ready-to-use reagent for the isolation of total RNA or the simultaneous isolation of RNA, DNA, and proteins from samples of human, animal, plant, yeast, bacterial and viral origin. TRI Reagent is capable of isolating RNA from even small amounts of starting material. TRI Reagent is a monophasic solution that includes phenol and guanidine thiocyanate to facilitate the immediate and most effective inhibition of RNase activity while cell lysis.

Storage and Stability
TRI Reagent is shipped at room temp and stable for 12 months when stored at 2-8ºC away from light.

Special Handling Instructions
TRI Reagent solution contains a corrosive liquid/poison (phenol) and an irritant (guanidine thiocyanate). Contact with TRI Reagent solution will cause burns and can be fatal. Put on the appropriate personal protective equipment (gloves, Lab coat, face shield, and safety goggles) when working with TRI Reagent solution. Avoid breathing vapor. Read the warning notice on the bottle and SDS. In case of contact: Immediately flush eyes or skin with a large amount of water for at least 15 minutes and seek medical advice if necessary.

Materials and Equipment Required (Not Provided)

- Nuclease-free Water or DEPC-treated water
- Homogenization apparatus (tissue and certain cells only)
- Cooling centrifuge (capable of reaching12,000 x g, 2-8ºC)
- Sterile, polypropylene tubes that can withstand 12,000 × g in the presence of phenol/chloroform.
- Chloroform (free of all additives, such as isoamyl alcohol)
- 100% isopropanol
- 75% ethanol

Starting Material

Sample type	Starting material	Sample lysis
Tissues	50–100 mg (some tissues may require <50 mg of tissue such as liver and kidney)	Add 1 ml of TRI Reagent.
Adherent cells	1×10^6 –1×10^7 cells	Aspire growth media. Then, add 100 µl of TRI Reagent per cm^2 of surface area.
Suspension cells	Animal, plant, or yeast: 5 –10×10^6 cells	Pellet the cells by centrifugation. Then, add 3 volumes of TRI Reagent to 1 volume of cell suspension.
	Bacteria: 1×10^7 cells	
Blood	Up to 300 µl anticoagulated blood	Add 0.75-1 ml of TRI Reagent

Handling instructions:

- Use proper aseptic techniques while working with RNA.
- Work with cold TRI Reagent (4ºC) for best results.
- Always wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the skin. Change gloves frequently and keep tubes closed whenever possible.
- When processing lipid-rich samples avoid withdrawing the top fat layer.

Procedures

After sample collection

I. Perform RNA isolation instantly to avoid degradation.
II. Quick-Freeze at –80°C or in liquid nitrogen until RNA isolation.

Note

I. The capacity of the tube should be at least twice the volume of the sample and Tri Reagent combined.
II. The volume of the sample should not be greater than 10% of the volume of Tri Reagent.

a. Tissues

1. Add 1 ml ice-cold TRI reagent to a sterile polypropylene centrifuge tube and drop the tissue into the solution.
2. Homogenize the tissue using a homogenizer, instantaneously and thoroughly, to create a homogeneous mix.

b. Cultured cells

1. Decant culture medium.
2. **For adherent cells,** add directly 1 ml TRI Reagent for each 10 cm^2 area (100 µl per cm^2) Alternatively, harvest monolayer adherent cells and wash using 1X PBS, then, add 100 µl TRI Reagent per cm^2.
   For Suspension cells, Pellet the cells by centrifugation. Then, add 3 volumes of TRI Reagent to1 volume of cell suspension. Pipette the lysate up & down several times to homogenize

c. Blood

1. Transfer up to 300 µl of blood sample to a 1.5 ml of RNase-free microcentrifuge tube
2. Add 3 volumes of TRI Reagent to each volume of liquid sample (3:1). Mix well by vortex.

3. Incubate the homogenate at room temp for 5 minutes to ensure the full dissociation of nucleoprotein complexes.

Stop point: Samples can be stored at 4°C overnight or –20°C for up to a year.

4. Centrifuge the lysate for 5 minutes at 12,000 × g at 4°C, then transfer the clear supernatant to a new tube. (Only if the sample is rich in fats, polysaccharides, proteins, and extracellular material)
5. Transfer 200 µl Chloroform for each 1 ml Tri Reagent required for lysis, cap the tube securely, and shake it thoroughly. (Do not vortex)
6. Incubate for 3 minutes.
7. Centrifuge for 15 minutes at 12,000 × g at 4°C.

Note: The mixture separates into a lower red phenol-chloroform, an interphase, and a colorless upper aqueous phase.

8. Transfer the colorless upper aqueous phase (containing RNA) by angling the tube at 45° and pipetting the solution to a new centrifuge tube.

Note: I. Avoid transferring any of the interphase or organic layer while removing the aqueous phase. II. Save the interphase and lower red organic phase from each sample, for DNA and protein isolation.

9. Optional: add 5–10 µg of RNase-free glycogen as a carrier to the aqueous phase.

Note: The glycogen is co-precipitated with the RNA but does not interfere with subsequent applications.

10. Add 500 µl isopropanol to the aqueous phase for each 1 ml Tri Reagent required for lysis.
11. Incubate for 10 minutes at 4°C.
12. Centrifuge for 10 minutes at 12,000 × g at 4°C.
13. Discard the supernatant with a micropipette.
14. Re-suspend the pellet in 1 mL of 75% ethanol per 1 ml Tri Reagent required for lysis.
15. Wash the RNA pellet in the ethanol by vortexing briefly.
16. Centrifuge for 5 minutes at 7500 × g at 4°C.
17. Discard the supernatant with a micropipette.
18. Vacuum or air dry the RNA pellet for 5–10 minutes.
19. Re-suspend the pellet in 20–50 µl of RNase-free or DEPC-treated water, 0.1 mM EDTA, or 0.5% SDS by passing the solution through a pipette tip several times.
20. Incubate sample at 55–60°C in a water bath or heat block for 10–15 minutes.
21. Check RNA concentration and integrity to ensure yield and quality.
22. Store RNA Samples at -20 °C

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