TRIpure Total RNA Extraction Reagent

Catalog# BPC1001

Size: 100 ml

Lot # Check on the product label

Description

TRIpure Reagent is a broad-spectrum total RNA extraction reagent. It is fast and easy to use, with bright colours and easy stratification. The reagent is used to extract total RNA from animal tissues, plant material, various microorganisms and cultured cells. The method provides good separation of small amounts of tissue (50-100mg) and cells (5 x 10^6) as well as large amounts of tissue (≥1g) and cells (>10^7). The samples were sufficiently lysed in TRIpure while being able to maximise RNA integrity. After centrifugation with chloroform, the solution is divided into three layers: an upper colourless aqueous phase, an intermediate layer and a lower red organic layer, with the RNA distributed in the supernatant layer. The extracted total RNA is free from protein and DNA contamination and can be used in a variety of routine molecular biology experiments such as RT-PCR, Real-time RT-PCR, Northern blot, Dot Blot, in vitro translation, etc. The RNA is then collected and precipitated in isopropanol to obtain total RNA.

TRIpure reagent facilitate the precipitation of a wide range of RNAs of different molecular weights and sizes from different species. For example, agarose gel electrophoresis of RNA extracted from rat liver and stained with ethidium bromide revealed many discrete high molecular weight bands between 7 kb and 15 kb (mRNA and hnRNA components), two dominant ribosomes of ~5 kb (28S) and ~2 kb (18S), and low molecular weight RNA between 0.1 and 0.3 kb (tRNA, 5S). When the extracted RNA is diluted with TE the A260/A280 ratio is ≥1.8. Note that with normal agarose gel electrophoresis, the 28S position is approximately 2 kb and the 18S position is approximately 1 kb, and the position varies considerably with different gel concentrations.

Kit Contents and Storage

Components	Size	Storage
TRIpure Reagent	100 ml	Store at 2-8°C for for 12 months

Reagents required But Not supplied:

1. Chloroform
2. Isopropanol
3. 75% ethanol (prepared with DEPC-treated water)
4. RNase free water or DEPC treated water.

Note: This product contains phenol, which is toxic and corrosive. It can cause poisoning, burns and other bodily harm if inhaled, in contact with the skin, swallowed, etc. Wear protective items such as protective clothing, gloves, eye protection, face shield etc. when using this product. In case of accidental contact, flush immediately with plenty of water and seek medical attention.

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- **Procedure**

Note: Wear gloves and eye protection when extracting RNA with TRIpure. Avoid contact with skin and clothing. Complete the procedure in a chemical fume hood. Avoid respiratory inhalation. Should be operated at room temperature of 15-30°C.

1. Homogenization

a. Plant tissue: Take fresh plant tissue and grind it thoroughly in liquid nitrogen or cut up the plant tissue and grind it rapidly directly in TRIpure, add 1 ml of TRIpure per 50-100 mg of tissue and mix well. Note: The sample volume should not normally exceed 10% of the TRIpure volume.

b. Animal tissue: Take fresh or -70°C frozen animal tissue and cut up as much as possible, add 1ml TRIpure per 30-100mg of tissue and homogenise with a homogeniser. Or grind in liquid nitrogen and add 1ml of TRIpure and mix.

Note: The sample volume should not normally exceed 10% of the TRIpure volume.

c. Monolayer cultures: remove as much residual culture fluid as possible and add 1 ml of TRIpure directly to a 3.5 cm diameter plate to cover and repeatedly blow the lysed cells. The amount of TRIpure required is based on the area of the plate rather than the number of cells (1 ml per 10 cm2). Inadequate amounts of TRIpure can result in DNA contamination in the extracted RNA.

Note: The fact that the walled cells do not completely detach from the culture flask (dish) does not mean that the lysis is incomplete, the cell membrane has actually broken open and all RNA has been released, so continue.

d. Cell suspension: Collect the cells by centrifugation. The cells should be lysed in TRIpure reagent by repeatedly blowing with a pipette. Add 1 ml of TRIpure per 5-10 x 10^6 animal, plant or yeast cells or per 1 x 10^7 bacteria. avoid washing cells before adding TRIpure as this increases the likelihood of mRNA degradation. Lysis of some yeasts and bacteria may require the use of a homogeniser.

e. Blood: We recommend the use of our TRIpure Reagent Liquid Sample, a TRIpure Reagent for whole blood or liquid samples, which is equivalent to TRIzol LS.

2. The homogenised sample is shaken vigorously and left at room temperature for 5 minutes to allow complete dissociation of the nucleoprotein bodies.

3. Optional step: Centrifuge at 12,000 rpm for 10 minutes at 4°C and remove the supernatant.

If the sample contains large amounts of protein, fat, polysaccharides or muscle, tuber nodules of plants etc., this can be removed by centrifugation. When processing samples of adipose tissue, the upper layer is heavily oiled and should be removed. The clarified homogenate is taken for the next step.

4. Add 0.2 ml of chloroform per 1 ml of TRIpure. Cap the tube tightly, shake vigorously for 15 seconds and leave it at room temperature for 2 to 3 minutes.

5. Centrifuge at 12,000 rpm at 4°C for 10-15 minutes at high speed. The RNA is present in the aqueous layer without exception. The volume of the aqueous layer is approximately 50-60% of the TRIpure volume added. (The organic and intermediate layers are protein and DNA, please contact us for extraction methods if required).

6. Transfer the aqueous layer to a clean centrifuge tube and add an equal volume of isopropanol. Mix upside down and leave at room temperature for 10 minutes.

The RNA precipitate is usually not visible before centrifugation and forms a gelatinous precipitate on the side and bottom of the tube after centrifugation.

7. Centrifuge at room temperature or 12,000 rpm at 4°C for 10 minutes and discard the supernatant.

8. Add 75% ethanol to wash the precipitate. Wash the precipitate with 1 ml of 75% ethanol for 1 ml of TRIpure used.

9. Centrifuge at room temperature or 12,000 rpm at 4°C for 3 minutes and discard the supernatant, taking care not to lose the RNA precipitate.

Note: The remaining small amount of liquid can be centrifuged briefly and then aspirated with a gun tip, taking care not to discard the precipitate.

10. Leave at room temperature for 2-3 minutes and allow to dry. Add 30-100μl RNase free water to fully dissolve the RNA and store the resulting RNA at -70°C to prevent degradation.

Note: Do not over-dry the precipitate as it may be difficult to dissolve.

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