TRIpure Reagent Total RNA Extraction Reagent

Item No. HRP303 50 ml
HRP303M 100 ml

Storage conditions:
Store in the dark at 2-8℃, valid for one year.
Important hint:
This product contains phenol, which is toxic and corrosive. If inhaled, touched with skin, swallowed, etc., it can cause poisoning, burns and other physical injuries. When using this product, you should wear protective items, such as protective clothing, gloves, goggles, face shields, etc. If you accidentally touch it, you should immediately rinse with plenty of water and go to the hospital for treatment.
Product description:
TRIpure Reagent is a broad-spectrum total RNA extraction reagent. The experiment operation is fast and convenient, the color is bright, and it is easy to layer. This reagent has a wide range of applications and can extract total RNA from samples such as animal tissues, plant materials, various microorganisms and cultured cells. This method is suitable for a small amount of tissue (50-100mg) and cells (5×106)
And a large number of tissues (≥1g) and cells (>107) have good separation results. The sample is fully lysed in TRIpure Reagent while ensuring the integrity of RNA to the utmost extent. After centrifugation with chloroform, the solution will be divided into three layers: the upper colorless water phase, the middle layer and the lower organic phase. RNA is distributed in the supernatant layer. After collecting the supernatant layer, the total RNA can be recovered by isopropanol precipitation. The extracted total RNA has good integrity, no protein and DNA pollution, and can be used in various molecular biology routine experiments, such as RT-PCR, Real-time RT-PCR, Northern blot, Dot Blot, in vitro translation, etc. TRIpure reagent can promote the precipitation of a variety of RNAs of different species and different molecular weights. For example, RNA agarose gel electrophoresis extracted from rat liver and stained with ethidium bromide showed many discontinuous high-molecular-weight bands (mRNA and hnRNA components) between 7 kb and 15 kb, two dominant ribose Body ~5 kb (28S) and ~2 kb (18S), low molecular weight RNA is between 0.1 and 0.3 kb (tRNA, 5S). When the extracted RNA is diluted with TE, its A260/A280 ratio is ≥1.8. Note that in the case of ordinary agarose gel electrophoresis, the position of 28S is about 2kb, and the position of 18S is about 1kb. The position of gels with different concentrations varies greatly.
Precautions:
1. After the sample is homogenized with TRIpure Reagent, it can be stored at -70°C for more than one month if it is not added immediately before adding chloroform. RNA precipitation stored in 75% ethanol can be stored at 2-8°C for one week, and at -20°C for one year. RNA has a short half-life and is easily degraded. It is recommended to perform follow-up experiments as soon as possible after extraction, such as reverse transcription into cDNA, Northern Blot, etc.

2. Bring your own reagents: chloroform, isopropanol (newly opened or special for RNA extraction), 75% ethanol (prepared with DEPC-treated water), RNase free water or DEPC-treated water.

RNA extraction operation steps: (please read the precautions before the experiment)
Tip: Wear gloves and goggles when extracting RNA with TRIpure. Avoid contact with skin and clothing. Complete the operation in a chemical fume hood. Avoid breathing in the respiratory tract. Unless otherwise specified, all operations should be at room temperature.
1. Homogenization
Plant tissue: Take fresh plant tissue and grind it thoroughly in liquid nitrogen 4 or cut the plant tissue into small pieces and grind it directly in TRIpure. Add 1ml TRIpure for every 50-100mg of tissue and mix well. Note: Generally, the sample volume should not exceed 10% of the TRIpure volume.
Animal tissue: Take fresh or frozen animal tissue at -70℃ and cut it as much as possible, add 1ml TRIpure for every 30-100mg tissue, and homogenize with a homogenizer. Or add 1ml of TRIpure after grinding in liquid nitrogen and mix well. Note: Generally, the sample volume should not exceed 10% of the TRIpure volume.
Monolayer cultured cells: Try to remove the remaining culture medium as much as possible, and directly add 1ml of TRIpure to the 3.5 cm diameter culture plate to cover and repeatedly pipette to lyse the cells. Determine the amount of TRIpure required based on the area of the culture plate rather than the number of cells (add 1ml per 10cm2). When the amount of TRIpure is insufficient, the extracted RNA may be contaminated with DNA.
note:
Adherent cultured cells: often cannot be completely detached from the culture flask (dish). This does not mean that the lysis is incomplete. At this time, the cell membrane has actually been completely ruptured and all RNA has been released, just continue the operation.
Cell suspension: Collect cells by centrifugation. Use a pipette to lyse the cells in the TRIpure reagent. Add 1ml of TRIpure for every 5~10×106 animal cells, plant or yeast cells or every 1×107 bacteria. Avoid washing cells before adding TRIpure, as that will increase the possibility of mRNA degradation. Rupture of certain yeasts and bacteria may require the use of a homogenizer.
Blood: It is recommended to use our company's TRIpure ReagentLS, which is dedicated to whole blood or liquid samples. TRIpure ReagentLS is the abbreviation of Liquid Sample. The homogenized sample was shaken vigorously and placed at room temperature for 5 minutes to completely dissociate the nucleoprotein bodies.
Optional step: Centrifuge at 12,000 rpm for 10 minutes at 4°C and take the supernatant.
If the sample contains more protein, fat, polysaccharide or muscle, tuber nodules of plants, etc. can be removed by centrifugation. The pellet after centrifugation contains the outer cell membrane, polysaccharides, and high molecular weight DNA, and the supernatant contains RNA. When processing adipose tissue samples, the upper layer is a lot of grease that should be removed. Take the clear homogenate and proceed to the next step.

2. Add 0.2ml chloroform per 1ml TRIpure Reagent. Close the tube cap tightly, shake vigorously for 15 seconds and place it at room temperature for 2 to 3 minutes.
3. High-speed refrigerated centrifugation at 12,000 rpm at 4°C for 10-15 minutes. After centrifugation, the mixture was divided into three layers: the lower organic phenol chloroform layer, the middle layer, and the upper colorless watery layer. RNA exists in the water layer without exception. The capacity of the water sample layer is approximately 50-60% of the capacity of the added TRIpure Reagent. (The organic layer and the middle layer are protein and DNA).
4. Transfer the water sample layer to a clean centrifuge tube and add an equal volume of isopropanol. Invert and mix well, then let stand at room temperature for 10 minutes.
RNA precipitation is usually not visible before centrifugation, after centrifugation, gelatinous precipitates are formed on the side and bottom of the tube.
5. Centrifuge at 12,000 rpm at room temperature or 4°C for 10 minutes, and discard the supernatant.
6. Add 75% ethanol to wash the precipitate. Wash the precipitate with 1 ml of 75% ethanol for every 1 ml of TRIpure Reagent used.
7. Centrifuge at 12,000 rpm at room temperature or 4°C for 3 minutes, discard the supernatant, and be careful not to lose the RNA pellet.
Note: The remaining small amount of liquid can be centrifuged briefly, and then sucked out with a pipette tip, being careful not to aspirate the precipitate. To
8. Leave at room temperature for 2-3 minutes and let dry. Add 30-100μl RNase free water to fully dissolve the RNA, and store the obtained RNA at -70°C to prevent degradation.
Note: Do not dry the precipitate too much, so as not to be difficult to dissolve.