*ClearBand TRUE*zol Reagent

100 ml

Cat No: TR100

Shipping : Ship at ambient temperature.
Storage : Store at 4-8°C

General Information

*ClearBand TRUE*zol Reagent is a ready-to-use reagent composed of phenol and a mixture of other components for the isolation of high-quality total RNA from various biological materials including animal and plant tissues, cells and bacteria.

Biological materials are homogenized or lysed in *ClearBand TRUE*zol Reagent and then separated into three phases: a clear upper aqueous phase with the RNA, a pink lower organic phase and an interphase, containing DNA and protein. RNA is purified by precipitation with isopropyl alcohol and then washed to remove impurities.

Purified RNA can be effectively used in routine downstream applications including cDNA synthesis, northern blotting, differential display, primer extension, and mRNA selection.

Protocol for Total RNA Isolation

1 mL of *ClearBand TRUE*zol Reagent is sufficient to isolate RNA and DNA from 1 x 10^7 cells or 100 mg of tissue material.

I. Homogenization

Tissue: Homogenize 50-100 mg of tissue materials in 1 mL of *ClearBand TRUE*zol Reagent. If samples have a high fat content, a layer of fat may accumulate at the top, which should be removed by centrifugation for 5 mins at 12000g at 4°C.

Plant tissue: Homogenize plant tissue materials in 1 mL of *ClearBand TRUE*zol Reagent. After homogenization, discard insoluble material by centrifugation at 12000g for 10 mins at 4 °C. Transfer the cleared homogenate to a new RNAse free microcentrifuge tube.

Cells grown in monolayer: Remove cell culture media and lyse cells directly in a cell culture plate or flask by adding 1 mL of *ClearBand TRUE*zol Reagent per 10 cm^2 area. Pipette the cell lysate several times to ensure sufficient cell disruption.

Cells grown in suspension: Pellet cells by centrifugation at 200g for 5 mins at room temperature. Lyse cells in 1 mL of *ClearBand TRUE*zol Reagent. Pipette the lysate up and down several times for complete homogenization.

Samples after homogenization can be stored at 4°C overnight or at -70°C for up to one year.

II. Phase Separation

1. Incubate samples for 5 mins at room temperature.
2. Add 0.2 ml of chloroform per 1 ml of *ClearBand TRUE*zol Reagent.
3. Carefully cap tubes and shake thoroughly by shaking for 15 seconds.
4. Incubate samples for 3 mins at room temperature.
5. Centrifuge samples at 12000g for 15 mins at 4°C.
6. The sample will separate into a pink organic phase, an interphase and a colorless upper aqueous phase that contains the RNA.

III. RNA Precipitation

1. Transfer the upper colorless aqueous phase very carefully to another RNAse free microcentrifuge tube without disturbing the interphase.
Use interphase and organic phase for DNA and protein isolation with protocols provided below.
2. Add 0.5 ml of isopropyl alcohol per 1 ml of *ClearBand TRUE*zol Reagent used.
3. Incubate for 10 mins at room temperature.
4. Centrifuge at 12000g for 10 mins at 4°C. Discard the supernatant. Total RNA will precipitate as a white pellet at the bottom of the tube.

IV. RNA Wash

1. Resuspend the pellet in 1 ml of 75% ethanol per 1 ml of *ClearBand TRUE*zol Reagent used.
2. Vortex samples and centrifuge at 7500g for 5 mins at 4°C.
3. Discard the supernatant with a micropipette.

V. Re-Dissolving the RNA

1. Air-dry the pellet and dissolve in DEPC-treated water by pipetting the solution up and down.
2. Store RNA at -70°C.
Note: Incubate for 10 minutes at 55-60°C if necessary, before storing the RNA at -70°C.

For further information;
ecotechbiotech.com
info@ecotechbiotech.com

Protocol for DNA Isolation

1. Remove any residual aqueous phase overlying the interphase.
2. Add 0.3 mL of absolute ethanol per 1 mL of *ClearBand TRUE*zol Reagent used for lysis. Carefully cap tubes and mix by inverting the tube several times.
3. Incubate for 2–3 minutes. Centrifuge for 5 minutes at 2000g at 4°C to pellet the DNA.
4. Transfer the phenol-ethanol supernatant to a new tube.
The supernatant is used for protein isolation.
5. Resuspend the pellet in 1 mL of 0.1 M sodium citrate in 10% ethanol, pH 8.5 per 1 mL of *ClearBand TRUE*zol Reagent used for lysis. Incubate for 30 minutes mixing occasionally by gentle inversion.
The DNA can be stored in sodium citrate/ethanol for at least 2 hours.
6. Centrifuge for 5 minutes at 2000g at 4°C.
7. Discard the supernatant with a micropipette. Repeat step 5 and 6 once.
8. Resuspend the pellet in 1.5–2 mL of 75% ethanol per 1 mL of *ClearBand TRUE*zol Reagent used for lysis.
9. Incubate for 10–20 minutes, mixing occasionally by gentle inversion.
The DNA can be stored in 75% ethanol at several months at 4°C.
10. Centrifuge for 5 minutes at 2000g at 4°C.
11. Discard the supernatant with a micropipette.
12. Vacuum or air dry the DNA pellet for 5–10 minutes.
13. Resuspend the pellet in 0.3–0.6 mL of 8 mM NaOH by pipetting up and down.
We recommend resuspending the DNA in a mild base because isolated DNA does not resuspend well in water or Tris buffer.
14. Centrifuge for 10 minutes at 12000g at 4°C to remove insoluble materials.
15. Transfer the supernatant to a new tube, then adjust pH as needed with HEPES. Proceed to downstream applications, or store the DNA at 4°C overnight. For longer-term storage at –20°C, adjust the pH to 7–8 with HEPES and add 1 mM EDTA.
16. Determine the DNA yield.

Protocol for Protein Isolation

1. Remove any residual aqueous phase overlying the interphase.
2. Add 0.3 mL of absolute ethanol per 1 mL of *ClearBand TRUE*zol Reagent used for lysis. Carefully cap tubes and mix by inverting the tube several times.
3. Incubate for 2–3 minutes. Centrifuge for 5 minutes at 2000g at 4°C to pellet the DNA.
4. Transfer the phenol-ethanol supernatant to a new tube.
5. Add 1.5 mL of isopropanol to the phenol-ethanol supernatant per 1 mL of *ClearBand TRUE*zol Reagent used for lysis. Incubate for 10 minutes.
6. Centrifuge for 10 minutes at 12000g at 4°C to pellet the proteins. Discard the supernatant with a micropipette.
7. Prepare a wash solution consisting of 0.3 M guanidine hydrochloride in 95% ethanol.
8. Resuspend the pellet in 2 mL of wash solution per 1 mL of *ClearBand TRUE*zol Reagent used for lysis.
9. Incubate for 20 minutes. Centrifuge for 5 minutes at 7500g at 4°C.
The proteins can be stored in wash solution for at least 1 month at 4°C or for at least 1 year at – 20°C.
10. Discard the supernatant with a micropipette. Repeat step 8 and 9 twice.
11. Add 2 mL of 100% ethanol, then mix by vortexing briefly. Incubate for 20 minutes.
12. Centrifuge for 5 minutes at 7500g at 4°C.
13. Discard the supernatant with a micropipette.
14. Air dry the protein pellet for 5–10 minutes.
15. Resuspend the pellet in 200 µL of 1% SDS by pipetting up and down.
To ensure complete resuspension of the pellet, we recommend incubation of the sample at 50°C in a water bath or heat block.
16. Centrifuge for 10 minutes at 10,000 × g at 4°C to remove insoluble materials.
17. Transfer the supernatant to a new tube. Measure protein concentration by *ClearBand* Bradford Reagent.

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