ClearBand *TRUE*zol LS Reagent

100 ml

Cat No: TRLS100

Shipping : Ship at ambient temperature.
Storage : Store at room temperature.

General Information

ClearBand *TRUE*zol LS 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 liquid biological materials of human, animal, plant, yeast, bacterial, and viral origin. *ClearBand* *TRUE*zol LS Reagent is not suitable for use with whole blood.

Biological materials are homogenized or lysed in *ClearBand* *TRUE*zol LS 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

0.75 mL of *ClearBand* *TRUE*zol LS Reagent is sufficient to isolate RNA and DNA from 0.25 ml liquid biological material.

I. Homogenization

Liquid biological material: Mix 0.75 ml of *ClearBand* *TRUE*zol LS Reagent with 0.25 ml of sample and homogenize the sample by passing the suspension several times through a pipette. If the sample volume is <0.25 ml, adjust the volume to 0.25 ml with water. The volume ratio of *ClearBand* *TRUE*zol LS Reagent to sample should always be 3:1.

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

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 LS Reagent.
3. Carefully cap tubes and shake thoroughly by shaking for 15 seconds.
4. Incubate samples for 3-15 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. Store the interphase and organic phase at 2-8 °C for subsequent isolation of the DNA and proteins.
2. Add 0.5 ml of isopropyl alcohol per 1 ml of *ClearBand* *TRUE*zol LS 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;
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Protocol for DNA Isolation

1. Remove any residual aqueous phase overlying the interphase.
2. Add 0.3 mL of absolute ethanol per 0.75 mL of *ClearBand TRUE*zol LS 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 0.75 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 0.75 mL of *ClearBand TRUE*zol LS Reagent used for lysis.
9. Incubate for 10–20 minutes at room temperature, 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 0.75 mL of *ClearBand TRUE*zol LS 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 LS Reagent used for lysis. Incubate for 10 minutes at room temperature.
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 0.75 mL of *ClearBand TRUE*zol LS 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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