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.
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;
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