MRE-3200K 200 ml

USER GUIDE: RNA Extraction Reagent

Storage at 2-8°C and keep away from light.

Description:

EBL RNA Extraction Reagent - is a fast, simple way of preparing RNA for RT-PCR, northern blots, RNase protection assays, and other molecular experiments requiring RNA. EBL RNA Extraction Reagent includes phenol and guanidine thiocyanate which effectively inhibits the RNase activity, while facilitates the isolation of a variety of RNA species of large or small molecular size. Following the addition of chloroform and subsequent centrifugation, the homogenate separates into an aqueous phase, an interphase and an organic phase. RNA is recovered in the aqueous phase with the addition of isopropyl alcohol, and is subsequently washed in ethanol and dissolved in RNase-free water. An optional DNase I treatment may improve certain downstream applications.

Applications:

Preparation of total RNA from a variety samples for RT-PCR, northern blots, and other molecular experiments requiring RNA.

Required materials not supplied:

0.2 ml of chloroform per ml of EBL RNA Extraction Reagent
0.5 ml of Isopropyl alcohol per ml of EBL RNA Extraction Reagent
75% ethanol (prepared in DEPC-treated water)
RNase-free water (DEPC-treated water)

To avoid introducing RNases, great care must be taken in handling the reagents and purified RNA samples. An RNase-free environment will yield the best results. Wear latex or vinyl gloves when handling reagents or RNA and change gloves frequently.

Isolate RNA Protocol:

Special Handling Instructions:
EBL RNA Extraction Reagent contains phenol and guanidine thiocynate. The reagent is flammable and can be fatal if ingested. When working with EBL RNA Extraction Reagent, wear gloves and goggles

1. Disrupt and homogenize the sample

• Cells grown in monolayer:

Remove the growth media and add 1 ml of EBL RNA Extraction Reagent to a 10 cm^2 dish. Pipet the lysate up and down several times to homogenize it.

• Cells grown in suspension:

Pellet the cells by centrifuging at 3,000 - 5,000 g for 2 minutes and immediately adding 1 ml of EBL RNA Extraction Reagent to 1 x 10^7 cultured mammalian. Pipet the lysate up and down several times to homogenize it.

•Frozen Tissues:

Grind the frozen tissues into fine powder with a mortar and a pestle in liquid nitrogen. Add 1 ml of EBL RNA Extraction Reagent to every 50 - 100 mg of the frozen tissue in the mortar and immediately homogenize until samples reagent thaw.

2. Incubate the lysate at room temperature for 5-10 minutes once the sample has been disrupted in EBL RNA Extraction Reagent, to allow the complete dissociation of nucleoprotein complexes.

3. Add chloroform into such RNA extraction reagent mixture in the ratio of 0.2 ml to 1 ml and agitate vigorously for about 15s. Do not vortex.

4. Incubate for 2-3 minutes at room temperature while periodically mixing the sample.

5. Centrifuge at 13,000 rpm for 15 minutes at 4°C.

6. Transfer the colorless upper RNA water phase into a new RNase-free tube.

Note: It is crucial that none of the interphase or organic phase be transferred with the aqueous phase. It is recommended to apply together RNA Separation Gel (MRE-3100G) to help locking RNA water phase.

7. Add 0.5 ml of isopropanol to the water phase. Mix thoroughly and then incubate at room temperature for 5-10 minutes.

8. Centrifuge with 13,000 rpm at 4°C for 10 minutes.

9. The RNA will appear as a white pellet on the side and bottom of the tube. Carefully discard the supernatant and wash the pellet with 75% ethanol.

10. Discard the liquid and vacuum the tube to dry the RNA pellet.

11. Resuspend the pellet in 20-50 µl of RNasefree water (DEPC-treated water). Pipet up and down a few times to completely resuspend the pellet. It may be necessary to incubate at 55–60°C for 10 minutes to completely dissolve the RNA pellet. The extracted RNA can be used immediately in downstream applications. Store RNA samples at -70°C. Avoid freeze-thaw cycles.