

LBzol™ Reagent

TZOL-001-100

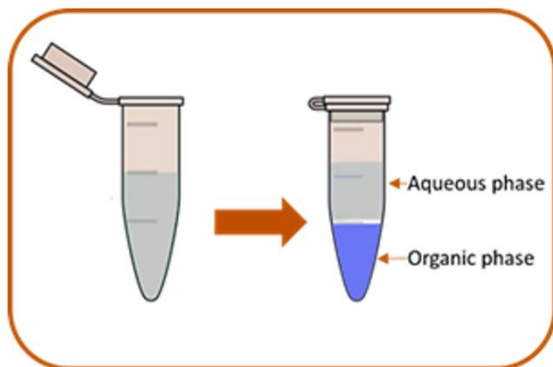
Introduction

LBzol™ Reagent is a ready-to-use reagent for the isolation of total RNA from various biological materials such as animal and plant tissues (rich in polysaccharides and proteoglycans), cell culture and bacterial cells.

This procedure is based on the sample lysis in cationic detergent guanidinium thiocyanate (GTC), followed by organic extractions and alcohol precipitation.

The biological sample is homogenized or lysed before being separated into three phases: an aqueous phase (upper), an organic phase (lower) and an interphase. The RNA remains in the aqueous phase, and its purification is followed by precipitation in isopropyl alcohol.

After removal of the aqueous phase, the DNA and proteins in the sample can be recovered by sequential precipitation. LBzol™ Reagent contains phenol and the mixture of other reagents to ensure optimal results.



Features

- ✓ Ready-to-use solution
- ✓ Quick isolation of high-quality total RNA, DNA and/or protein from a single sample
- ✓ Performs well with large or small amounts of tissue or cells

Applications

- ✓ Purified RNA is ideal for any downstream application such as RT-PCR, in vitro translation, Northern blotting, RNase protection assays or dot blot hybridization
- ✓ Purified DNA can be used for PCR and Southern blotting
- ✓ Purified protein can be used for Western blotting

Quality control

Each lot is tested in accordance with internal procedures. For Research Use Only.

Storage conditions

Store at 2-8°C, protect from light for up to 12 months.

Instructions for RNA isolation

Homogenization

✓ Tissues

Homogenize tissue samples in 1 ml of LBzol™ per 50-100mg of tissue. For small quantities of tissue (1-10 mg), add 800 µl of LBzol™.

OPTIONAL: Following homogenization, insoluble material is removed by centrifugation at 12000 x g for 10 minutes at 4°C. Transfer the cleared homogenate to a fresh tube

✓ Cells grown on monolayer

Lyse cells directly in a culture dish or flask by adding 1ml of LBzol™ per 10 cm² growth area, pipette the cell lysate several times to ensure sufficient cell disruption.

✓ Cells grown in suspension

Pellet cells at 200 x g for 5 minutes at room temperature. Lyse cells with 1 ml of LBzol™ per 5 x 10⁶ cells and pass the lysate several times through a pipette tip. For small quantities of cells (10² – 10⁶), lyse cells in 800 µl of LBzol™.

Note: At this stage, samples can be stored for at least one month at -70°C

Phase Separation

1. Incubate samples for 5 minutes at room temperature
2. Add 0.2 ml of chloroform (not supplied) per 1 ml of LBzol™ used
3. Cap tubes securely and shake vigorously by hand for 15 seconds
4. Incubate samples for 3 minutes at room temperature
5. Centrifuge samples at 12000 x g for 15 minutes (or 2600 x g for 30 minutes) at 4°C
6. The sample will separate into a pale-yellow organic phase, an interphase and a colorless upper aqueous phase that contains the RNA

RNA Precipitation

7. Transfer the aqueous phase very carefully, without disturbing the interphase to another tube
8. Precipitate the RNA by mixing with cold isopropyl alcohol (not supplied). Use 0.5 ml of isopropyl alcohol per 1 ml of LBzol™ used
9. Incubate samples for 10 minutes at room temperature
10. Centrifuge at 12000 x g for 10 minutes (or 2600 x g for 30 minutes) at 4°C

RNA Wash

11. Remove the supernatant
12. Wash the pellet once with 75% ethanol (not supplied), adding at least 1 ml of ethanol per 1 ml of LBzol™ used
13. Vortex samples and centrifuge at 7500 x g for 5 minutes at 4°C

Re-dissolving the RNA

14. Air-dry the pellet and dissolve in PCR grade water by pipetting the solution up and down
15. Incubate for 10 minutes at 60°C if necessary
16. Store RNA at -70°C