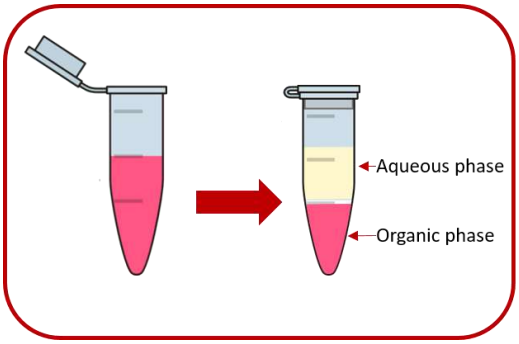
Data sheet

PRImeZOL™ Reagent

Cat. No: AN1100
Cat. No: AN1102

Description

PRImeZOL™ 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.

PRImeZOL™ Reagent contains phenol and the mixture of other reagents to ensure optimal results.

	Contents	
	AN1100	**AN1102**
PRImeZOL™ Reagent	100 ml	2X100 ml

Storage conditions

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

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 to internal procedures.

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INSTRUCTIONS FOR RNA ISOLATION

1. Homogenization

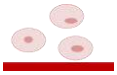
Tissues 

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

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 **PRImeZOL™** per 10 cm^2 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 **PRImeZOL™** per 5 x 10^6 cells and pass the lysate several times through a pipette tip. For small quantities of cells (10^2 – 10^6), lyse cells in 800 µl of **PRImeZOL™**.

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

2. Phase Separation

1. Incubate samples for 5 minutes at room temperature.
2. Add 0.2 ml of chloroform (not supplied) per 1 ml of **PRImeZOL™** 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.

3. RNA Precipitation

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

4. RNA Wash

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

5. Re-dissolving the RNA

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

For Research Use Only.
CAUTION: Not for human or animal therapeutic or diagnostic use.

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