

# *EXTRAzol*

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**blirt**



# EXTRAzol

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

Using **EXTRAzol** a 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. The highly effective RNase inhibitory property of **EXTRAzol** protects the integrity of the RNA during lysis and results in the isolation of high-quality material (high RIN and S28/S18 values). **EXTRAzol** contains phenol and the mixture of other reagents to ensure optimal results.

1 ml of **EXTRAzol** is sufficient to isolate total RNA from  $1 \times 10^7$  cells or 100 mg of tissue. The isolation method is fast and easy to carry.

RNA isolation obtained by using **EXTRAzol** provides reliable results in the gene expression based studies using such applications as synthesis of cDNA, real-time RT-PCR, microarrays, hybridization assays, *in vitro* translation.



## Features

- 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
- ready-to-use solution

## 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

# EXTRAzol

## PROTOCOL FOR RNA ISOLATION USING EXTRAzol

### Reagents required (not supplied with EXTRAzol)

- chloroform
- isopropyl alcohol (chilled)
- 75% ethanol (in DEPC-treated water)
- DEPC-treated water or PCR-grade water

### I. Homogenization

#### Tissue

Homogenize tissue samples in 1 ml of **EXTRAzol** per 50-100mg of tissue. For small quantities of tissue (1-10 mg), add 800 µl of **EXTRAzol**. For samples of fat tissue, a layer of fat may accumulate at the top, which should be removed.

#### Plant tissue

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 **EXTRAzol** per 10 cm<sup>2</sup> 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 **EXTRAzol** per  $5 \times 10^6$  cells and pass the lysate several times through a pipette tip. For small quantities of cells ( $10^2$ – $10^6$ ), lyse cells in 800  $\mu$ l of **EXTRAzol**.

**Note:** at this stage, samples can be stored for at least one month at  $-70^\circ\text{C}$ .

### II. Phase Separation

1. Incubate samples for 5 minutes at room temperature.
2. Add 0,2 ml of chloroform per 1 ml of **EXTRAzol** 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  $4^\circ\text{C}$ .
6. The sample will separate into a pale yellow organic phase, an interphase and a colorless upper aqueous phase that contains the RNA.

### III. 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. Use 0.5 ml of isopropyl alcohol per 1 ml of **EXTRAzol** 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.

### IV. RNA Wash

1. Remove the supernatant.
2. Wash the pellet once with 75% ethanol, adding at least 1 ml of ethanol per 1 ml of **EXTRAzol** used.
3. Vortex samples and centrifuge at 7500 x g for 5 minutes at 4°C.

### V. Re-dissolving the RNA

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

## Quality control

Each LOT of the **EXTRAzol** reagent is tested in accordance to internal procedures.

## Safety information



### Danger

H314, H301, H311, H331, H341, H373

P264, P270, P280, P260, P271, P284, P301+P310

P330, P302+P352 P312, P361 P363, P304+P340 P310,

P301+P330+P331, P303+P361+P353, P304+P340 P310,

P305+P351+P338, P308+P313, P403+233, P405

**H314** Causes severe skin burns and eye damage. **H301** Toxic if swallowed. **H311** Toxic in contact with skin. **H331** Toxic if inhaled. **H341** Suspected of causing genetic defects. **H373** May cause damage to organs through prolonged or repeated exposure. **P264** Wash hands thoroughly after handling. **P270** Do not eat, drink or smoke when using this product. **P280** Wear protective gloves/protective clothing/eye protection/face protection. **P260** Do not breathe dust/fumes/gas/mist/vapours/spray. **P271** Use only outdoors or in a well-ventilated area. **P284** In case of inadequate ventilation wear respiratory protection. **P301+P310 P330** IF SWALLOWED: Immediately call a POISON CENTER/ doctor. Rinse mouth. **P302+P352 P312** IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. **P361 P363** Take off immediately all contaminated clothing. Wash contaminated clothing before reuse. **P304+P340 P310** IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. **P301+P330+P331** IF SWALLOWED: rinse mouth. Do NOT induce vomiting. **P303+P361+P353** IF ON SKIN (or hair): take off immediately all contaminated clothing. Rinse skin with water/ shower. **P304+P340 P310** IF INHALED: remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. **P305+P351+P338** IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. **P308+P313** If exposed: call a POISON CENTER or doctor / physician. **P403+233** Store in a well ventilated place. Keep container tightly closed. **P405** Store locked up.

# EXTRAzol

| Component | EM30-100 | EM30-200  |
|-----------|----------|-----------|
| EXTRAzol  | 100 ml   | 2x 100 ml |

## Storage & shipping

### Storage conditions

Store at +4°C

### Shipping conditions

Shipping at room temperature

 for research use only

## Expiry

The information on the label