# RNA Extraction

## Workflow

**RNA Extraction** → **cDNA synthesis (RT-PCR)** → **Real-Time PCR**

In biological research, RNA isolation is a crucial step in the journey to discovery and is necessary for any assays which analyze the presence or quantity of mRNA transcripts, e.g. Real-Time PCR.

## Specimen

**Type: Cells in culture, Tissues** (Samples must be stored in a -80°C freezer)

## Materials

### Reagents (including catalog No. and storage conditions)

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| **Chloroform** | Sigma, Catalog # C243<br>It is stored at room temperature in the flammable liquids chemical cabinet. |
| **TRIzol reagent** | Invitrogen Life Technologies, Catalog # 15596-026.<br>It is stored in a refrigerator in a dark container. |
| **Nuclease Free Water** | Fisher, Catalog # BP2484-50 |
| **75% Ethanol (-20°C)** | Sigma, Catalog #E7023 |
| **Isopropanol** | Sigma, Catalog # I0398<br>It is stored at room temperature in the flammable liquids chemical cabinet. |

## Equipment and supplies:

Refrigerated Microcentrifuge

Centrifuge tubes

Pipettors - Adjustable, 1-10 μL, 5-20 μL, 20-200 μL, 100-1000 μL

Nanodrop

Vortex Mixer

Water/Dry Bath

Ice

## Safety (Warning and Biohazard consideration):

TRIzol reagent is very toxic when in contact with skin and if swallowed. It will cause burns. Be sure to wear a lab coat, gloves and safety glasses when working with TRIzol reagent. If in contact with skin, wash immediately with plenty of soap and water. Work in a chemical fume hood.

## Procedure:

## Steps:

**1. Homogenization:**

**Tissues:** Homogenize tissue samples in 1 ml of TRIZOL reagent per 50 to 100 mg of tissue.

**Cells:** Remove the media (for adherent cells just remove whole media covering the cells but for the suspend cells first centrifuge the media in 130g for 5 minutes then remove the supernatant).

Add 400 µL Trizol reagent (for each plate containing 5×105 cells) and shake it for 20 min in room temperature to disrupting cells.

Incubate the homogenized sample for 10 minutes at room temperature to permit the complete dissociation of nucleoprotein complexes. Centrifuge to remove cell debris. Transfer the supernatant to new tube.

**2. Phase Separation:**

Add 0.2 ml of chloroform per 1 ml of TRIZOL Reagent. Cap sample tubes securely. shake the tube by hand for about 15 sec. and incubate them at room temperature for 2 to 3 minutes. Centrifuge the samples at no more than 12,000 x g for 15 minutes at 4C. Following centrifugation, the mixture separates into:

- ✓ colorless upper aqueous phase
- ✓ interphase
- ✓ lower red, phenolchloroform phase

RNA remains exclusively in the aqueous phase. Transfer upper aqueous phase carefully without disturbing the interphase into fresh tube.

**3. RNA Precipitation:**

Precipitate the RNA from the aqueous phase by mixing with cold isopropyl alcohol. Add 0.5 ml of isopropyl alcohol per 1 ml of TRIZOL Reagent used for the initial homogenization. Incubate samples at room temperature for 10 minutes and centrifuge at 12,000x g for 10 minutes at 2 to 4 C. The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

**4. RNA wash:**

Remove the supernatant completely. Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of TRIZOL Reagent used for the initial homogenization. Mix the samples by inversion and centrifuge at no more than 7,500 xg for 5 minutes at 4 C. Remove all leftover ethanol.

**5. Redissolving RNA**:

Air-dry RNA pellet for 5-10 minutes. Do not dry the RNA pellet by centrifuge under vacuum. Resuspend the RNA pellet in 50 µL of nuclease-free water by pipetting up and down gently and incubating in a 50-60°C water/dry bath for 10 minutes

**6.** Use nanodrop instrument for measuring RNA concentration and purity. (The 260/280 ratio should be greater than 1.8)

## References:

1. Chomczynski P, Mackey K. (1995) Short technical report. Modification of the TRIZOL reagent procedure for isolation of RNA from Polysaccharide-and proteoglycan-rich sources. Biotechniques 19(6): 942-5.
2. TRIzol reagent literature, Invitrogen Life Technologies.

## شناسنامه سند: CoreLab.101

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