MediRes Corp.

USER GUIDE **G3013-100ML**

RNA Extraction Solution

Product #: G3013-100ML
Quantity: 100 mL

Product Description

- RNA extraction solution is a ready-to-use reagent, designed to isolate high quality total RNA from cell and tissue samples.
- This product utilizes a monophasic solution of phenol, guanidine isothiocyanate, and other proprietary components which facilitate the isolation of a variety of RNA species of all sizes (miRNA to large RNA products).
- RNA extraction solution maintains RNA integrity due to highly effective inhibition of RNase activity while disrupting cells and dissolving cell components during sample homogenization.
- Purified RNA products can be directly used for subsequent molecular biology applications.

Storage

- Store at 4°C in a dark environment for up to 12 months.

Components Provided

RNA Extraction Solution 100 mL

User Supplied Components

Nuclease Free H_20
Chloroform
Isopropanol
75% Ethanol
Centrifuge and rotor capable of reaching 12,000 x *g* and 4 ^{0}C

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Important Preparatory Notes (PLEASE READ CAREFULLY)

1. Perform all steps at room temperature (20–25°C) unless otherwise noted.
2. Use cold **RNA Extraction Solution** if the starting material contains high levels of RNase, such as spleen or pancreas samples.
3. Use disposable, individually wrapped, sterile plasticware and sterile, disposable RNase-free pipettes, pipette tips, and tubes.
4. Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin; change gloves frequently, particularly as the protocol progresses.
5. Always use proper microbiological aseptic techniques when working with RNA.

Samply Lysis

Tissues:

1. Add 1 mL of **RNA Extraction Solution** per **50 – 100 mg** of tissue.
2. Homogenize sample until there are no visible tissue clumps.
3. Centrifuge at **12,000 x *g*** for **10 min** at **4°C** to separate the tissue fragments.
4. Transfer the supernatant to a new centrifuge tube.

Adherent Cells:

1. Remove growth media and wash cells with **ice-cold 1X PBS**.
2. Remove PBS wash thoroughly.
3. Add 0.25 to 1 mL of **RNA Extraction Solution** per **1×10^5—10^7 cells** directly to the culture dish to lyse the cells.
4. Pipet the lysate up and down several times to homogenize.
5. Transfer the sample to a new centrifuge tube.
6. Let the sample stand at **room temperature** for **5 min** before proceeding.

Cell Suspension:

1. Pellet the cells by centrifugation and discard the supernatant thoroughly.
2. Add **0.25 to 1 mL** of **RNA Extraction Solution** per **1×10^5—10^7 cells**.
3. Pipet the lysate up and down several times to homogenize.
4. Transfer the sample to a new centrifuge tube.
5. Let the sample stand at **room temperature** for **5 min** before proceeding.

RNA Purification Protocol

1. Add **380 µL** of **chloroform** per **1 mL** of **RNA Extraction Solution**.
2. Vortex vigorously for 15 s, and let sample stand at **room temperature** for **3 min**.
3. Centrifuge sample at **12,000 x *g*** for **15 min** at **4°C**.
4. Carefully transfer the upper colorless aqueous phase to a new centrifuge tube.
   - ***Note:*** *Approximately **500-550µL** of aqueous solution can be extracted per **1 mL** of **RNA Extraction Solution**.*
   - ***Note:*** *the interphase and organic phases can be saved for DNA or protein isolation.*
5. Precipitate RNA by adding **550µL** of **isopropanol**. Gently invert several times.
6. Place tube at **-20°C** for **15 minutes** to enhance the precipitation of the RNA.
7. Centrifuge at **12,000 x *g*** for **10 min** at **4°C**.
8. Total RNA precipitate forms a white gel-like pellet at the bottom of the tube. Discard the supernatant using a micropipettor.
9. Wash the pellet by adding **1 mL** of **75% ethanol** and mix by inversion.
10. Centrifuge at **12,000 x g** for **5 min** at **4°C**.
11. Discard the supernatant using a micropipettor.
12. Briefly centrifuge (**5,000 x g** for **3-5 s**), and carefully remove the remaining liquid using a micropipettor.
13. **Air-dry** the RNA pellet by opening the centrifuge tube for **5 min**.
14. After the RNA pellet has slightly dried, add 20 – 50 µL of nuclease-free water to fully dissolve the RNA. Mix by pipetting or vortex.
    - ***Note:*** *Be careful not to let the RNA dry too much, or the pellet is difficult to dissolve.*
    - ***Optional:*** *Incubate on a thermomixer for 10 min at 1000 rpm and 37^{0}C.*
15. Determine the RNA yield and purity.
16. Store the purified RNA at -80°C for long-term storage. Avoid repeated freeze-thaw of the RNA.

NOTES:

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