

RNA ISOLATION PROTOCOL



Uniformed Services University
Department of Microbiology and Immunology
4301 Jones Bridge Road
Bethesda, MD 20814

Used by the Laboratory of William C. Gause, Ph.D.

TISSUE PREPARATION

1. Place 100 mg tissue in a polypropylene tube with 1.5 mL RNAzol B.
2. Homogenize the sample thoroughly.
3. Snap-freeze the sample in liquid nitrogen and store at -70°C .

RNA EXTRACTION

1. Thaw frozen homogenates ~5 minutes in a 37°C water bath.
2. Add 0.15 mL of a 24:1 mixture of chloroform/isoamyl alcohol for every 1.5 mL of homogenate.
3. Shake the samples vigorously for 15 seconds.
4. Allow the samples to sit on ice for 5 minutes.
5. Centrifuge the samples for 15 minutes in a refrigerated centrifuge (4°C) at $14,000g$.
6. Transfer the aqueous (top) layer to another tube, avoiding the white interphase layer.
7. Store on ice.

RNA PRECIPITATION

1. Add a volume of cold isopropanol that is equal to the volume of the aqueous phase.
2. Mix the tubes gently.
3. Store the tubes on ice for 15 minutes or in -20°C overnight.
4. Centrifuge the samples for 15 minutes at 4°C and $14,000g$. The RNA should form a whitish/yellowish pellet at the bottom of the tube.

RNA WASHING

1. Decant the isopropanol.
2. Wash the RNA pellet by adding 1mL cold 75% ethanol.
3. Resuspend the RNA pellet by vortexing.
4. Centrifuge the sample for 10 minutes at 4°C and $14,000g$. Repeat the wash step again.
5. Decant the ethanol and dry the pellets by air or under vacuum. Care should be taken not to overdry the pellet under vacuum.

RNA QUANTIFICATION

1. Solubilize the RNA pellets in 20-50 μL of ddH₂O.
2. Quantify the product spectrophotometrically by measuring the $\text{OD}_{260}/\text{OD}_{280}$. The $\text{OD}_{260}/\text{OD}_{280}$ ratio should be 1.8 or above. If the ratio is low, the sample should be reextracted.
3. Store samples at -70°C .