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**RNA Isolation Protocol-Cultured Cell Samples**

V3-112202

**Required Reagents:**

Chloroform  
Isopropanol  
75% Ethanol (cold)  
Monophasic Isolation Reagent  
RNA Precipitation Solution  
    1.2M NaCl  
    0.8M Disodium Citrate  
DNase (RNase-free)  
Ultra-pure water  
Liquid Nitrogen

**Sources**

Disodium Citrate (CAS#6132-05-4)

<u>Manufacturer</u>	<u>Cat. Num.</u>
<i>Sigma</i>	<i>35908-4</i>
<i>Fluka</i>	<i>71635</i>
<i>Riedel</i>	<i>25134</i>

DNase (RNase-free)

<u>Manufacturer</u>	<u>Cat. Num.</u>
<i>Qiagen</i>	<i>79254</i>
<i>Ambion</i>	<i>2222</i>
<i>Promega</i>	<i>M6101</i>

Monophasic Isolation Reagents:

<u>Name</u>	<u>Manufacturer</u>
<i>RNA Wiz</i>	<i>Ambion</i>
<i>Trizol</i>	<i>Gibco/Invitrogen</i>
<i>TRI Reagent</i>	<i>Sigma</i>

**Solutions**

250ml RNA Precipitation Solution

17.532g      NaCl  
52.622g      disodium citrate  
to 250ml with Ultra-pure water

## Protocol

- For attached cells, remove the culture media and lyse cells using 1ml monophasic isolation reagent per  $1 \times 10^7$  cells.

*Approximately 3mls of monophasic isolation reagent are required for each T175 flask.*

- For cells grown in suspension, harvest the cells by gentle centrifugation and homogenize cells in 1ml monophasic isolation reagent per  $1 \times 10^7$  cells.
- Pass the monophasic isolation reagent through a 22-ga needle by drawing into a sterile syringe three times.
- Incubate the homogenized sample for 5 minutes at RT to allow complete dissociation of protein complexes.
- Add 0.2ml chloroform per milliliter of monophasic isolation reagent. Mix samples vigorously by inversion or vortexing.
- Separate the two phases by centrifugation at 10,000xg for 15 minutes at 4°C and carefully transfer the upper aqueous phase to a fresh tube

*Great care should be taken to avoid transferring any of the material at the interface between the aqueous and organic phases. Any residual material transferred will contaminate the RNA sample with DNA or protein.*

- Precipitate the RNA from the aqueous phase by adding 0.25 volume of isopropanol and 0.25 volume of RNA precipitation solution per ml of the monophasic isolation reagent used at the start of the protocol. Mix thoroughly and incubate 10 minutes at RT.
  - Collect the precipitated RNA by centrifugation at 12,000xg for 10 minutes at 4°C.
  - Wash the RNA pellet with 1ml of 75% Ethanol for each milliliter of monophasic isolation reagent used at the start of the protocol. Mix gently and centrifuge at 7,500xg for 5 minutes at 4°C.
  - Remove the supernatant taking care not to disturb the pellet. Allow the pellet to air dry for 10-15 minutes. Do not allow the pellet to completely dry out.
  - Resuspend the pellet in 200-400ul of Ultra-pure water. Spec the RNA at OD 260 to determine the ug amount of RNA. Add appropriate volume of 10x DNase reaction buffer to a final concentration of 1x.
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- Add 0.4U of Promega DNase per ug of RNA and incubate at 37°C for 30 minutes. (Note: Volumes may have to be adjusted depending on amount of DNase used. Amounts of DNase as low as 0.1U per ug RNA have been used with success.)
- Following incubation, add 4ml monophasic isolation reagent (Trizol) and mix by inversion. (Note: Trizol volume must be a minimum of 10X the digestion volume.)
- Add 0.8ml chloroform, centrifuge at 10,000xg for 15 minutes at 4°C and transfer the aqueous phase to a fresh tube. (Note: Adjust chloroform if necessary; use 0.2ml chloroform per ml of Trizol.)
- Add 0.25 volumes isopropanol and 0.25 volumes RNA precipitation solution, mix vigorously and incubate 10 minutes at RT (precipitation may be enhanced by incubating the solution at -20°C for 1 hour to overnight).
- Recover the precipitated RNA by centrifugation at 12,000xg for 15 minutes at 4°C.
- Wash the RNA pellet with 4ml of cold 75% ethanol and centrifuge at 7,500xg for 5 minutes at 4°C.
- Carefully decant the supernatant and allow the pellet to air dry for 10-15 minutes (do not allow the pellet to dry completely).
- Resuspend in water or 10mM Tris pH 7.5 and quantitate by UV 260/280.

**NOTE:** For further information on general handling of RNA see the Molecular Cloning Laboratory Manual by Sambrook. For more information on this protocol specifically, see Chapter 7, Protocol 2.

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