Extraction and Purification of Total RNA using Trizol or Tri Reagent

Susan L. Gottshall[1], Saban Tekin[2], and Peter J. Hansen[2]

[1]Purdue Pharma Inc. and [2]Dept. of Animal Sciences, University of Florida

Reagents and Equipments

TRIzol Reagent (Life Technologies cat# 15596-026) or TRI reagent (Sigma cat # T-9424)
DEPC (RNase free) water or 0.5% SDS solution in DEPC treated water
Chloroform (Fisher)
Isopropyl alcohol (2-Propanol) (Fisher)
75% Ethanol (in DEPC treated water)
Sterile or RNase treated pipette tips, microcentrifuge tubes, and pestles or motorized homogenizer.
Microcentrifuge

1. Homogenization for Cell Suspensions

a) Place 1 ml aliquots of the cell suspension in sterile RNase free 1.5 ml microcentrifuge tubes.
b) Centrifuge for 1 minute to pellet the cells.
c) Pour off the supernatant.
d) Add 1 ml of TRIzol or TRI reagent to the tubes.
e) Lyse cells by repetitive pipetting.
f) Centrifuge homogenate at 12000 x g for 10 minutes at 4 ^{o}C.
g) Transfer the homogenate in a sterile microcentrifuge tube.
h) If this RNA will be used for RT-PCR, repeat steps f and g twice.

Modification for tissues

a. Add 1 ml of TRIzol or TRI reagent to every 50-100 mg of tissue. Sample volume should not exceed 100 µl. If you using 1.5 -2 ml microcentrifuge tube and pestle for homogenization, start with 500 µl of TRIzol or TRI reagent, then add remaining 500 µl.
b. Homogenize the samples using a sterile or RNAse free plastic, glass pestles or power homogenizer and tubes.

Modification for monolayers

a. Lyse cells directly in a culture dish by adding 1 ml of TRIzol or TRI reagent to a 3.5 cm diameter dish.
b. Pass the cells through a pipette several times.

2. Phase Separation

a. Incubate samples (from 1g) for 5 minutes at room temperature.
b. Add 0.2 ml of chloroform to each tube.
c. Cap each tube. Shake samples vigorously by hand for 15 seconds.
d. Incubate samples for 5 minutes at room temperature.
e. Centrifuge samples for 15 minutes at 12,000 x g at 4^{o}C.

3. RNA Precipitation

a. Transfer the upper aqueous phase to a fresh tube.

b. Add 0.5 ml of isopropyl alcohol to precipitate RNA. If this RNA will be used for RT-PCR, first add 50 µl isopropyl alcohol, mix, incubate the samples at room temperature for 5 minutes and centrifuge at 12,000 x g for 10 minutes at 4°C. Transfer the sample in a new tube.
c. Incubate for 5-10 minutes at room temperature.
d. Centrifuge for 10 minutes at 12,000 x g at 4°C. The RNA will form a pellet on the side or bottom of the tube.

4. RNA Wash

a. Discard the supernatant.
b. Wash pellet with 1 ml 75% ethanol.
c. Mix sample by vortexing. The RNA pellet may float.
d. Centrifuge at 12000 x g for 5 minutes at 4°C. If this RNA will be used for RT-PCR repeat steps a,b and c twice.
e. RNA pellet may be stored in ethanol at -70°C for months.

5. Redissolving the RNA

a. Remove supernatant.
b. Air dry the pellet for 5-10 minutes. Do not completely dry out the pellet.
c. Dissolve pellet in 30 to 60 µl RNase free water or 0.5% SDS by passing the solution through a pipette tip and incubating for 10 minutes at 55-60°C.

6. Determination of RNA Concentration and Purity

a. Take 2 to 5 µl RNA sample from the original stock, diluted with 998 or 995 µl RNase free water in a 1.5 ml microcentrifuge tube. This will give you 500 or 200 time dilution of the RNA sample.
b. Pipet 1 ml RNAse free water in a clean cuvette and read absorbance as blank.
c. Pipet the diluted RNA sample in to a clean cuvette and read absorbance at 260 nm and 280 nm.
d. Use the formula below to determine RNA Concentration of the original sample:

$$[\text{RNA } \mu g/\mu l] = A_{260} \times 33 \times \text{dilution factor} / 1000$$

e. To determine the purity of the RNA sample, calculate ratio of A260/A280. (Ratios between 1.7 to 2 represent good RNA)

Preparation of RNase-free water

a. Measure water into RNase-free glass bottles.
b. Add 1 ml of 0.1% (v/v) diethylpyrocarbonate (DEPC) to 1000 ml water.
c. Let stand overnight.
d. Autoclave.

Note: RNase free DEPC treated water is Biotecx brand (cat # BL-5611).

Note: If using 0.5% SDS solution to resuspend the RNA. It must be prepared in RNase free water.

modified 05-15-2008