Isolation of total RNA with TRI-reagent (Tissue <100mg)

Sample preparation

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16 April 2009

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We used the NuGO Standard Operating Procedure (SOP) number 50 produced by the University of Aberdeen. Details of the SOP are available via the web link:

http://www.nugo.org/frames.asp?actionID=39147&action=loginFromPP

Recommended: 1 ml TRI-reagent per 50-100 mg tissue. See manufacturers protocol.

INTRODUCTION

TRI Reagent is a complete and ready-to-use reagent for isolation of total RNA or the simultaneous isolation of RNA, DNA and proteins from samples of human, animal, plant, yeast, bacterial and viral origin. The technique performs well with small and large quantities of tissue or cultured cells, and allows simultaneously processing of a large number of samples.

REAGENTS/EQUIPMENT

RNase/DNase free 2.0/1.5/0.5 ml eppendorf tubes
Rnase/DNase free pipette tips of appropriate size
TRI-reagent (Sigma, T-9424) – protect from light, store 4^0C
Chloroform
Isopropanol
Ethanol 100 %
0.1 % Diethyl Pyrocarbonate (DEPC) -treated ddH_2O, store 4^0C
Liquid Nitrogen
Ultra Turrax tissue homogeniser
Refrigerated Centrifuge (up to 12,000 x g)
Ice bucket
Gloves
Eye protection
Fume hood

Timer

0.1 % DEPC water

500 µl DEPC
500 ml millipore H_2O

Cover and leave to stir overnight in fume hood. Autoclave next morning and store at 4 ^{o}C.

METHOD

1. Keep samples frozen during weighing (work in liquid nitrogen). Weigh out ~ 50 - 100 mg tissue into a 2 ml eppendorf tube.

2. Add 0.5 ml TRI-reagent to frozen tissue and keep on ice. Homogenise (using the ultra turrax) for 30 sec at level 5.

3. Add an additional 0.5 ml TRI reagent. Can use this to wash off excess sample from the homogeniser down into the tube.

4. Wash the homogeniser between each sample. Set up four 25 ml plastic tubes with 10-15 ml 0.1% DEPC water. Switch on the probe in first tube and leave for several seconds making sure probe is well washed, switch off and move onto next tube, repeat. Repeat with remaining tubes. Change 0.1% DEPC water regularly. Wipe probe before starting next sample.

5. Incubate the homogenised sample for 5 min on ice to permit the complete dissociation of nucleoprotein complexes.

6. Add 200 µl chloroform. Mix by inverting the tube for 15 sec. Leave for 5 min on ice and spin at 12,000 x g for 15 min at 4 ^{o}C.

7. Transfer the aqueous phase (upper phase) to a new 1.5 ml tube and precipitate the RNA with 500 µl Isopropanol. If extracting DNA also see appropriate protocol – do not throw away interphase of lower phase, can keep at 4^{o}C overnight.

8. Incubate the samples for ~ 2 hours or overnight at -20 ^{o}C.

9. Centrifuge at 12,000 x g for 10 min at 4 ^{o}C.

10. Remove the supernatant by pipetting and wash the pellet with 1 ml **75% ethanol-DEPC** water. Gently flick the tube to dislodge to pellet allowing

the ethanol to circulate round the pellet. Incubate for 10 min on ice to remove trace of TRI-reagent, and then spin at 5,000 x g for 5 min at 4 ^{o}C.

11. Remove the supernatant carefully by pipetting, and allow the pellet to air dry for ~15 min on ice. Another quick spin will help to remove residual liquid.

12. Resuspend pellet in 40 – 70 µl DEPC-water.

13. Check the RNA by:

    i) **NanoDrop** to determine RNA concentration:
       2µl RNA + 2 µl DEPC-water in a 0.5 ml tube
    ii) **RNA gel analysis** for RNA quality:
       5µg/well
    iii) **Agilent** for RNA quality:
       Neat or 1:10 dilution (DEPC-water) – *if using RNA for micro array or real time PCR, RNA will need to be DNase treated before Agilent*.

12. Store the RNA at –80 ^{o}C. Aliquot the samples in RNase/DNase free tubes in appropriate volumes depending on next stage. This will help reduce freeze/thaw cycles and RNA denaturation.