Total RNA isolation (Guanidinium Thiocyanate-Phenol-Chloroform Extraction)

Experimental Considerations

1) This method is fast and works well for preparation of total RNA. We typically use total RNA for quantitative S1 nuclease analysis of endogenous (or in vitro) gene expression, and this method works well for this purpose.
2) The lysis buffer (i.e. the Guanidinium Thiocyanate-Phenol) is quite caustic and preparation can be hazardous. Therefore, we generally purchase this from Molecular Research Center (Tel# 888-841-0900, Web Site http://www.mrcgene.com/) under the name "TRI Reagent" (Cat# TR118, $95/100ml). If you expect to use a lot of this reagent, you may want to prepare it [see below or Chomczynski and Sacchi, Analytical Biochemistry **162**, 156-159 (1987)]

Protocol

- After harvesting cells, add an approximately equal pellet volume of 1X PBS and suspend cells [Note: If you're doing a time course experiment, you can freeze your pellets (on dry ice) and prepare RNA from all of them at the same time. If you freeze your pellets, don't let them thaw (and don't add 1X PBS) prior to adding TRI Reagent. Simply, suspend the frozen pellet in TRI Reagent (see next step) vigerously and as quickly as possible].
- Add 1ml TRI Reagent (for between 10^5 and 3 x 10^7 cells) and suspend cells immediately (see note**).
- Incubate at room temperature for 5-15 minutes.
- Add 200ul $CHCl_3$, vortex vigorously, 10-20 seconds.
- Incubate at room temperature for 5 minutes.
- Spin in microfuge (high speed, 15 minutes).
- Transfer aqueous phase into a new tube
- Add 0.5ml isopropanol, mix, and keep at room temperature for 10 minutes.
- Spin in microfuge (high speed, 20 minutes).
- Take off (or aspirate) the aqueous/isopropanol solution.
- Wash with 800 ul 70% EtOH (stored at –20°C)[i.e. add 70% EtOH, vortex, spin 5 minutes (high speed in microfuge) and take off EtOH]
- Dry in speedy vac and suspend RNA in 50-250ul high quality H_2O (i.e. distilled/deionized [if you're concerned about RNAses in the H_2O, you can pass it through a nitrocellulose filter prior to use since nitrocellulose binds well to small amounts of protein (RNAse)]). Quantitate and keep at –20°C (keep at –70°C if possible thawing due to heavy usage of –20°C is a potential issue).

** Note: The most important issue in RNA preparation is the speed in which the entire cell pellet is dissolved once the lysis has begun. Incomplete or slow lysis of the cell pellet causes the release of RNAses from subcellular compartments without fully inactivating the RNAse itself. This results in significant RNA degradation. Therefore, once you've added the lysis solution, pipette up and down vigorously until solution is homogeneous. In addition, the number of cells that are suspended in a given volume of TRI Reagent influences how well the cells become lysed. Do not try to suspend more than 3 x 10^7 cells per ml of TRI Reagent otherwise poor quality RNA can be expected. Conversely, if you're suspending less than approximately 2 x 10^6, the amounts of material used for the entire protocol can be halved (we typically use this amount for a 100mm plate of adherent cells).

Solutions

TRI Reagent (aka = AGPC (Acid Guanidinium Thiocyanate-Phenol-Chloroform) solution

To make the AGPC (or TRI Reagent) Solution, you will need the following starting solutions:

- 4M Guanidinium thiocyanate
[To minimize handling, 250 g guanidinium thiocyanate (Fluka) was dissolved in the manufacturer's bottle without weighing with 293 ml distilled H_2O]
- 0.75 M Sodium citrate pH 7.0
- 2 M Sodium Acetate pH 4.0
- 10% Sarcosyl (1g/10ml H_2O and filter)
- Phenol (nucleic acid grade, Sigma) (Melt phenol at 65^oC, add 0.1% w/v hydroxyquinoline and saturate with H_2O)

21ml of AGPC (TRI Reagent)

- Mix 10 ml 4M guanidinium thiocyanate, 352ul 0.75M sodium citrate (pH 7.0) and 528ul 10% sarcosyl
- Add 76 µl 14.3 M ß-mercaptoethanol -Sigma- [total final volume=10.9 ml].
- Place 10 ml of this mixture in a new tube, add 1 ml 2M sodium acetate (pH 4.0) and 10 ml water-saturated phenol. This is the final AGPC solution (This solution is good for at least 2 months at 4^oC).

105ml of AGPC (TRI Reagent)

- Mix 50 ml 4M guanidinium thiocyanate, 1.76ml 0.75M sodium citrate (pH 7.0) and 2.64ml 10% sarcosyl
- Add 381 µl 14.3 M ß-mercaptoethanol -Sigma- [total final volume=54.5 ml].
- Place 50 ml of this mixture in a new tube, add 5 ml 2M sodium acetate (pH 4.0) and 50 ml water-saturated phenol. This is the final AGPC solution (This solution is good for at least 2 months at 4^oC).