Isolation of total cellular RNA from brain tissue

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Equipment and reagents

- Dounce homogenizer or Polytron
- RNase-free equipment and instruments
- Brain tissue
- TRIzol™ reagent (Gibco BRL)
- DEPC-treated ddH_2O, SDS, ethanol, chloroform, isopropanol

Method

1 Remove the brains and isolate the tissue(s) of interest using sterile, RNase-free equipment and instruments. A sterile disposable Petri plate placed on ice can be used as an RNase-free surface for dissection. The tissue is then flash frozen by immersion in liquid nitrogen and stored in cryovials in liquid nitrogen prior to RNA extraction. As high quality RNA is essential for successful differential display, all tissue from an experimental set is stored in liquid nitrogen and the RNA samples are processed simultaneously to reduce the variability among the different RNA samples.

2 Total RNA is isolated using TRIzol™ reagent[a]. The frozen tissue is added to a Dounce homogenizer or centrifuge tube containing 1.0 ml of TRIzol per 50 mg of tissue. Homogenize the tissue using the Dounce homogenizer or, more conveniently, a Polytron until the sample is uniformly dissociated. Rapid homogenization of the brain tissue in TRIzol minimizes RNA degradation. When processing multiple samples, the Polytron unit can be cleaned between samples using a series of washes including a rinse in ddH_2O, 1% (w/v) SDS, ddH_2O, 70% (v/v) ethanol, and ddH_2O. The ddH_2O used for rinsing and preparation of SDS and ethanol should be DEPC-treated.

3 Add 200 μl of chloroform for each 1.0 ml of TRIzol used for homogenization. Shake vigorously for 15 sec and incubate for 3 min at room temperature. Centrifuge the homogenate at 12 000 g for 15 min at 4 °C.

4 Carefully remove the colourless, upper aqueous phase avoiding the material that has collected at the interface. Place the aqueous phase containing the RNA in a clean microcentrifuge tube and add 0.5 ml of isopropanol per 1 ml of the TRIzol reagent used for homogenization. Usually, after phase separation and

centrifugation, 600 μl of aqueous phase is recovered. Mix the solution and allow the RNA to precipitate at room temperature for 15 min. Centrifuge at 12 000 g for 15 min at 4 °C. Remove the supernatant and wash the RNA pellet twice using 1.0 ml of 75% ethanol. The samples are vortexed after the addition of each ethanol wash and centrifuged at 7500 g for 3 min at 4 °C to ensure that the pellet has sedimented before the alcohol is removed. A brief centrifugation will collect any residual ethanol in the tube. The last drops of ethanol can be removed using a 26 gauge needle on a syringe.

5 The RNA is allowed to dry for approximately 10 min at room temperature. Protracted periods of air drying or drying the RNA pellet under vacuum will make the resuspension of the RNA difficult. After drying, the RNA samples are resuspended in 1 μl of ddH_2O per 1 mg of tissue sample, vortex mixed, placed on ice for 10 min, heated at 65 °C for 10 min and placed on ice before storage.

6 Determine the concentration of RNA in the sample spectrophotometrically. Usually the A_{260}/A_{280} ratio of the RNA is ~1.6 following TRIzol extraction. Although this ratio is lower than expected for pure RNA, it does not interfere with subsequent conversion of the mRNA to cDNA.

7 Store the total RNA at –70° C.

Notes

a There are many commercial kits available to isolate total RNA. We have found that TRIzol is the most reliable method for isolating RNA from all brain tissues and spinal cord.