DNase treatment of total cellular RNA to remove contaminating genomic DNA

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Reagents

- Dounce homogenizer or Polytron
- TRIzol™ reagent (Gibco BRL)
- ddH_2O, SDS, ethanol, chloroform, isopropanol
- 10 × One-Phor-All Plus buffer (Pharmacia)
- RNasin™ (Promega) (40 U/µl)
- RQ1 RNase-free DNase (Promega) (1 U/µl)

Method

1. To DNase-treat the RNA samples, combine 10 µg of RNA, 5 µl of 10 × One-Phor-All Plus buffer, 1 µl of RNasin™ (40 U/µl), 1 µl of RQ1 RNase-free DNase (1 U/µl), and DEPC-treated ddH_2O to a final volume of 50 µl.
2. Incubate the samples at 37 °C for 30 min.
3. Extract the DNase-treated RNA samples with 100 µl of TRIzol reagent as described in Isolation of total cellular RNA from brain tissue to remove the added DNase. The volume of the recovered aqueous phase is approx. 100 µl and, therefore, 84 µl of isopropanol is added to the aqueous phase to precipitate the RNA.
4. After two 70% (v/v) ethanol washes and brief drying, the DNase-treated RNA is resuspended in 9 µl of DEPC-treated ddH_2O, the concentration of RNA determined spectrophotometrically, and the concentration of RNA in solution adjusted to 500 ng/µl. The 260/280 ratio of the DNase treated RNA is usually between 1.8–1.9 after the second TRIzol extraction.

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