Preparation of Torula Yeast RNA for Hybe Solutions

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[Abstract] *In situ* hybridization in zebrafish embryos frequently suffers from high background signal. Torular yeast RNA is often added to reduce the non-specific binding that leads to the high background signal. Without appropriate preparation, torular yeast RNA can also add background to the staining. This method is an easy and quick way to clean torular RNA for Hybe solution used in *in situ* hybridization.

Materials and Reagents

1. Torula RNA (RNA from torula yeast, type VI) (Sigma-Aldrich, catalog number: R6225, 100 g)
2. TE (Life Technologies, Invitrogen™, catalog number: 12090-015)
3. RNase-free H_2O (Life Technologies, Ambion®, catalog number: AM9932)
4. 3 M NaOAc
5. Ethanol
6. Phenol
7. Chloroform
8. RNase-free 80% ethanol

Equipment

1. Standard tabletop centrifuges
2. 50 ml Falcon tubes

Procedure

1. Add 1 g of torula RNA powder to each 50 ml Falcon tube.
2. Add 10 ml of phenol, then 15 ml TE to each tube.
3. Vortex very hard until powder dissolves.
4. After all tubes are dissolved, spin using table-top centrifuge for 10 min (spin as fast as possible).

5. Remove top phase to a clean 50 ml Falcon tube.
6. Do 1 phenol/chloroform extraction (1:1).
7. Do 1 chloroform extraction.
8. Add 1 ml 3 M NaOAc and 30 ml 200 proof ethanol to supernatent. Let stand for 5 min.
9. Spin 20 min at full speed to pellet the RNA.
10. Dump supernatent.
11. Do an RNAse-free 80% ethanol rinse to remove salts.
12. Spin for 10 min.
13. Remove supernatent and let pellet dry.
14. Resuspend pellet in 15 ml RNase-free water.
15. Measure the concentration on the spec (I usually do 1:100 and 1:2,000 dilution to get the OD between 0.1 and 0.9).
16. Increase the volume to 20 mg/ml final concentration.
17. Aliquot into 15 ml Falcon tubes and store at -20 °C.

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