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Phenol-Chloroform Extraction for dsRNA Purification

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Protocol status: Working
We use this protocol and it's working

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Abstract

This is the final step in preparing injectable constructs for RNA interference. This protocol starts with a 20 uL T7-polymerase transcription reaction. We use New England Biolab's HiScribe kit. Our template cDNA has been prepared with T7 promoter regions at each 5' end, enabling RNA transcription in both directions. After dsRNA has been extracted, pelleted, and resuspended in pure nuclease-free water, we will melt the strands apart and allow them to reanneal slowly to ensure that we have plenty of properly double-stranded RNA.

The protocol is based on the manufacturer's manual for the HiScribe kit, modified with information from the MegaScript T7 RNA transcription kit manual (Ambion).

Materials

MATERIALS

- Isopropanol
- Chloroform **Fisher Scientific Catalog #**BP1145-1
- Phenol, (Carbolic acid) Double distilled for Molecular Biology **Bio Basic Inc. Catalog #**PD0252.SIZE.500g
- 70% Ethanol
- nuclease free water
- Ammonium acetate solution for molecular biology, 7.5 M **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A2706
- 1.5 ml reaction tube **Eppendorf**

Our ammonium acetate is 7.5 M stock. If only 5 M stock is available, adjust the volumes in the first two steps to accomodate 15 uL of 5M ammonium acetate in place of 13.33 uL of 7.4M ammonium acetate.

Safety warnings

Wear gloves and lab coat. Phenol and chloroform should be handled under a chemical fume hood.

Adjust salt conditions

1 Add 166.6 uL of nuclease free water to each PCR tube.
Move 187 uL of liquid to new, labeled 1.5 mL microcentrifuge tube. 1m

2 Add 13.33 uL of ammonium acetate to each tube. 1m

Extract RNA

3 Add 200 uL of acid phenol/chloroform (50:50) to each tube. Shake vigorously for 15 seconds. 1m

4 Centrifuge at 4C for 5 minutes at 12,000 * g or greater 6m

5 Move aqueous phase (top) to new tube (about 200 uL). 2m

6 Add an equal volume chloroform to the aqueous phase. Shake vigorously for 15 seconds. 30s

7 Centrifuge at 4C for 5 minutes at 12,000 * g or greater 6m

8 Move aqueous phase (top) to new tube 2m

Precipitate RNA

9 Add an equal volume of isopropanol. 30s

10 Incubate at room temperature for 10 minutes 10m

11 To pellet: Spin at 4C for 20 minutes at 12,000 * g or greater 21m

12 Remove isopropanol and Wash pellet with 75% ethanol in nuclease free water 2m

13 Spin at 4C for 5 minutes at 7,500 * g 6m

14 Remove all ethanol from pellet and let air dry (for about 15 minutes) 15m

Melt and anneal dsRNA

15 Resuspend dried pellet in 50 uL of nuclease free water. 1m

16 Heat resuspended dsRNA to between 65 and 80 degrees C for 5 minutes 5m

17 Allow to cool at room temperature for 10 minutes 10m

18 Place on ice. 15s