**Preparation of Buffer Saturated Phenol for RNA Extractions**

References:


- Sambrook, J., E. F. Fritch, and T. Maniatis, 1989. Molecular Cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, New York.
- Farrell Jr., R. E., 1993. RNA Methodologies: A Laboratory Guide for Isolation and Characterization. Academic Press, San Diego.


Materials:

- Redistilled Phenol, molecular biology grade: Stored in aliquots at -20°C.
- 8-hydroxyquinoline
- 0.5 M Tris·Cl (pH 8.0)
- 0.1 M Tris·Cl (pH 8.0)
- TE buffer
  10 mM Tris·Cl (pH 8.0), 1 mM EDTA
- Buffer A
  50 mM NaOAc, 10 mM EDTA

**NOTES:**
Phenol is volatile and caustic.
- PREPARE THE PHENOL IN THE FUME HOOD.
- WEAR GLOVES AND EYE PROTECTION.

All solution should be RNase free. Use RNase free glassware and plasticware. Follow the general guidelines in Sambrook et al., 1989 for the preparation of RNase free solutions and labware.

Procedure:

Aliquoting the phenol for storage:
1. Heat a water bath to 65°C in the fume hood. Place the bottle of phenol in the fume hood to warm to room temperature.
2. Place the bottle of phenol in the 65°C water bath to melt the crystals.
3. Once the phenol has melted, aliquot it into 15 or 50 ml polypropylene tubes or 100 ml glass bottles using a RNase free glass pipette. Seal the individual aliquots and wrap them in aluminum foil. Store at -20°C.

Preparation of TE buffered Phenol:
1. Melt an aliquot of phenol in a 65°C water bath.
2. Add 8-hydroxyquinoline to a final concentration of 0.1 % w/v to the phenol. Mix to dissolve the 8-hydroxyquinoline.
3. Add an equal volume of 0.5 M Tris·Cl (pH 8.0) to the phenol. Mix for 15 minutes. Return the bottle to the 65°C water bath. Allow the phases to separate (this will take approximately 5 minutes). Siphon off the top layer and discard.

4. Add an equal volume of 0.1 M Tris·Cl (pH 8.0) to the phenol. Repeat the procedure as in Step 3.
5. Repeat the extractions with 0.1 M Tris·Cl (pH 8.0) until the aqueous phase is ~pH 7.8 (measure with pH paper).
6. Add an equal volume of TE buffer to the phenol. Repeat the procedure as in Step 3. Leave a ~1 cm layer of TE over the phenol.
7. The buffer saturated phenol may be stored at 4°C for periods up to 2 months for RNA work. The phenol can be used up to 6 months for DNA work.

**Note:** Phenol will be lost during the preparation of the TE buffered phenol. Start with at least 2.5 X the final volume of phenol that you will need.

Preparation of Buffer A saturated phenol:

1. Melt an aliquot of redistilled phenol in a 65°C water bath.
2. Add 8-hydroxyquinoline to a final concentration of 0.1 % w/v to the phenol. Mix to dissolve the 8-hydroxyquinoline.
3. Add an equal volume of Buffer A. Mix for 15 minutes. Return the bottle to the 65°C water bath. Allow the phases to separate (this will take approximately 5 minutes). Siphon off the top layer and discard.
4. Repeat Step 3 a total of 3 times.
5. The buffer saturated phenol may be stored at 4°C for periods up to 2 months.

**Note:** Very little phenol will be lost during the preparation of the Buffer A saturated phenol. Start with at least 1.2 X the final volume of phenol you will need.