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## BUFFERING PHENOL

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**When you are using more than a few ul of phenol you must work in the fume hood.**

**This is protocol is a perfect example of using too much to be safe anywhere other than the fume hood.**

### Safety Notes:

- ◆ Phenol is nasty stuff. It denatures proteins so that is what it will do to the proteins that make up the skin on your hands and arms, etc. **Wear gloves, lab coat and safety glasses--NO EXCEPTIONS.** Refer to the MSDS for phenol for more information.
- ◆ Hydroxyquinoline is nasty too. It can irritate the skin and eyes and is a possible mutagen. Wear gloves, safety glasses and lab coat. Read the MSDS. Luckily it comes as fairly large crystals and has a high vapor pressure which means you can weigh it out CAREFULLY but without a mask.

Start with an unopened 1 L bottle of liquefied phenol from stores (88% phenol).

Add 1 gram of 8-hydroxyquinoline to a 2 liter flask and pour in the phenol. The phenol will turn yellow when the hydroxyquinoline dissolves--it is added as an anti-oxidant and scavenges free radicals.

Add 500mLs of 50mM Tris base. Stir up the two phases either by swirling the flask by hand or with a magnetic stirrer (remember to take all precautions when you retrieve the stirring bar later).

Allow the phases to separate and aspirate off the aqueous phase. Make sure you don't accidentally aspirate any phenol in the process as fish don't like it. It doesn't matter if you can't get all the aqueous phase off each time.

Repeat twice with 50mM Tris-CL pH8.0

Dispense into foil wrapped 50mL falcon tubes-- label them properly and store at -20 C. Store the aliquot that is in use in a brown bottle at 4 °C.