

## Phenol Preparation (Buffering)

### Procedure:

- (1) Remove the crystalline phenol from the  $-20^{\circ}\text{C}$  freezer and thaw it at  $60-65^{\circ}\text{C}$ .
- (2) Add desired volume of phenol to an appropriate sized bottle.  
(If you want/need 250-500ml of equilibrated phenol, use a 1000ml bottle.)
- (3) Add an equal volume of **10X TE** to the phenol.
- (4) Shake vigorously. Allow the layers to separate. This may take a while.
- (5) Aspirate off the aqueous (the top) layer. Do this in the fume hood.
- (6) Repeat with a second equal volume of 10X TE.
- (7) Add an equal volume of **1X TE** to the phenol.
- (8) Repeat with a second equal volume of 1X TE.
- (9) Leave a small layer of 1X TE above the phenol after the final aspiration.
- (10) Check the pH of the TE by dropping  $\sim 10\ \mu\text{l}$  onto pH paper. It should be  $\sim\text{pH } 8$ ; if it is still too high, perform additional TE equilibration steps.

### Notes:

Phenol does not last at  $4^{\circ}\text{C}$  indefinitely. If it has been at  $4^{\circ}\text{C}$  more than 3 months throw it away, if old phenol is used to prepare DNA, you will lose your DNA!

For short-term storage keep a convenient volume of a working stock at  $4^{\circ}\text{C}$ .  
For long-term storage; i.e., indefinite periods of time, store the phenol in the  $-20^{\circ}\text{C}$ , simply remove a convenient aliquot as you need it.

Phenol is a corrosive. It will cause severe burns if you get it on your skin. Use common sense and caution. Wear gloves, goggles and a lab coat and work in the fume hood when working with large quantities of phenol. If you spill it on yourself, wash the skin area well. If it has penetrated your clothing, remove your clothing immediately and wash your skin with water. If the burns are extensive, get help and go to the emergency room.

**Materials needed:**

(1) Crystalline phenol: JT Baker; VWR cat # JT2858-1

(2) 10X TE (T<sub>100</sub>E<sub>10</sub>)

<u>Stock</u>	<u>500ml Volume</u>
1M Tris <sub>(8.0)</sub>	50.0ml
0.5M EDTA	10.0ml
ddH <sub>2</sub> O	440ml

(3) 1X TE (T<sub>10</sub>E<sub>1</sub>)

<u>Stock</u>	<u>500ml Volume</u>
1M Tris <sub>(8.0)</sub>	5.0ml
0.5M EDTA	1.0ml
ddH <sub>2</sub> O	440ml

(4) Bottle

(5) An aspirator (a side arm Erlenmeyer flask set up) in the fume hood.

(6) Liquid waste container for the aspiratant