

PREPARATION OF ORGANIC REAGENTS

Phenol

Many batches of commercial, liquified phenol can be used without redistillation. However, batches that are pink or yellow and all crystalline phenol must be redistilled at 160°C to remove contaminants that cause breakdown or crosslinking of RNA and DNA. Liquified and redistilled phenol should be stored at -20°C in small aliquots, preferably under nitrogen gas.

As needed, phenol is removed from the freezer, allowed to warm to room temperature, and melted at 68°. 8-Hydroxyquinoline is then added to a final concentration of 0.1%. This yellow compound is an antioxidant, a partial inhibitor of RNase, and a weak chelator of metal ions (Kirby 1956). In addition, the yellow color provides a convenient way to identify the phase.

The melted phenol is then extracted several times with an equal volume of buffer (usually 1.0 M Tris [pH 8.0], followed by 0.1 M Tris [pH 8.0] and 0.2% β -mercaptoethanol), until the pH of the aqueous phase is >7.6. The phenol solution can be stored at 4°C under equilibration buffer for periods of up to 1 month.

Caution

Phenol is highly corrosive and can cause severe burns. Safety glasses and gloves should be worn. Any areas of skin that come into contact with phenol should be rinsed with a large volume of water and washed with soap and water. Do *not* use ethanol.

Chloroform:Isoamyl Alcohol (24:1)

A mixture of chloroform and isoamyl alcohol (24:1 v/v) is used to remove proteins from preparations of nucleic acid. The chloroform denatures proteins while isoamyl alcohol reduces foaming during the extraction and facilitates the separation of the aqueous and organic phases.

Neither reagent requires treatment before use. The mixture is stable and may be stored in closed bottles at room temperature.

Ether Saturated with Water

Ether is used to extract traces of phenol or other organic substances from aqueous solutions of nucleic acid. Traces of ether remaining after the extraction may be removed easily by heating to 68°C or by blowing a gentle stream of nitrogen gas over the sample. The ether is saturated with water to prevent loss of water from the sample during extraction and to inhibit the formation of free radicals, which form during storage and may damage DNA.

Mix an equal volume of ethyl ether (anhydrous) and distilled water in a screw-capped bottle. Shake well (tightly capped) and store at room temperature in a chemical hood. The ether is the upper of the two phases.

Caution

Ether is highly volatile and extremely flammable and should be worked with and stored in an explosion-proof hood.