Product Information

Phenol
for molecular biology

Catalog Number P1037
Storage Temperature 2–8 °C

CAS RN 108-95-2
Synonyms: Carbolic acid, Hydroxybenzene

Product Description
Molecular Formula: C₆H₆O
Molecular Weight: 94.11
Melting Point:¹ 140.85 °C
Boiling Point:¹ 1182 °C
Extinction coefficient:² $E_{\text{mM}}$ (ethanol) = 1.45 (276 nm), 1.91 (271 nm), 6.03 (218 nm)
Pka:³ 10.0 (25 °C)

This product is designated as Molecular Biology grade and is suitable for molecular biology applications.

Phenol is used in classic DNA extractions for removing protein. The best phenol for nucleic acid extractions is freshly distilled. If phenol becomes discolored, it is very likely oxidized and unsuitable for nucleic acid work. Oxidation products make DNA resistant to hydrolysis by DNase I. One such oxidation product is o-hydroxy-biphenyl. Inhibition arises from direct action on the DNA rather than on the enzyme, due to hydrogen bonding between bases of the nucleic acid. Several hydroxy biphenyls, including this one, have been studied.⁴

In an extraction for removing protein using phenol saturated with a buffer, at pH 8 or higher, DNA and RNA are more soluble in the upper, aqueous-rich layer leaving the proteins at the interface. At a pH below 7.0, the RNA remains soluble in the aqueous phase, but the DNA collects at the interface with the protein.

The DNA is removed from the aqueous layer with increasing efficiency as the pH is lowered with a maximum efficiency at pH 4.5. The phenol-rich phase does not separate well, so chloroform is added to force a cleaner separation of the organic and aqueous phases. Sometimes isoamyl alcohol is added as an antifoaming agent. A second extraction with just chloroform removes the residual phenol from the aqueous phase.

To prepare a phenol:chloroform:isoamyl alcohol reagent for DNA isolation, the phenol should initially be melted in a 45–50 °C water bath, and then the other reagents can be added.⁵ Hydroxyquinoline is typically added to this mixture as an antioxidant, but can be omitted, if necessary. However, the solution will last longer if it is added. In addition, the liquified phenol can be saturated with buffers to adjust the pH to the required value.

To accurately measure the pH of a buffered phenol solution, the following procedures should be followed:⁵

- For saturated phenol solutions (no chloroform nor isoamyl alcohol present), mix 2 ml of the organic phase (buffered phenol) with 5 ml absolute methanol and 13 ml of water. A single phase should result.
- For phenol:chloroform:isoamyl alcohol solutions, mix 2 ml of the organic phase with 6 ml of absolute methanol and 10 ml of water.

The resulting mixtures should be read with a pH meter against a calomel (mercury/mercuric chloride) reference electrode. A silver/silver chloride reference electrode will interfere with the accurate reading of Tris-containing solutions. Use of pH strips will not give accurate measurements. This method has an accuracy of 0.2 pH units.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
This product is soluble in water (50 mg/ml).
References
1. The Merck Index, 14th ed., Monograph # 07241.