Acid Phenol:Chloroform
MB Grade

Store at 4°C or –20°C.

Catalog #:	AM9720
Amount:	100 mL
Product Description:	Acid phenol:chloroform, premixed with isoamyl alcohol (125:24:1 phenol:chloroform:isoamyl alcohol).
Appearance:	Clear colorless liquid
Chemical Analysis:	Purity ≥99.0% pH 4.3–4.7
Caution:	**Phenol is very corrosive and will severely burn the skin. Safety precautions such as gloves, protective eyewear, a lab coat, and working in a fume hood are critical.**
Storage Conditions:	4°C or –20°C. Store in amber or foil-wrapped bottles, and do not open frequently, to avoid oxidation and breakdown products. To prolong the shelf life, store aliquots in 50 mL tubes at –20°C.

USER INFORMATION

General Information:

Phenol is a crystalline solid at room temperature. It is normally either saturated with an aqueous buffer or dissolved in chloroform to make it liquid. Phenol extraction is a commonly used method for deproteinization of nucleic acids [1, 2, 3]. Most proteins are more soluble in phenol than in the aqueous phase. Conversely, nucleic acids are more soluble in the aqueous phase. Centrifugation of the mixture will yield two phases; the lower phase is the organic phase and will contain the protein, usually as a white flocculent at the interface. The upper aqueous phase will contain nucleic acids.

Phase partitioning of nucleic acids is pH dependent [4, 5]. At pH 4–6 DNA will be retained in the organic phase and interface, leaving the RNA in the aqueous phase. Isolation of RNA [5, 6] from biological material is often done with an acid-phenol [6, 7]. For DNA isolation, a pH of 7.5–8.0 is required, and both DNA and RNA will partition into the upper aqueous phase. The pH dependence of DNA phase partitioning makes it necessary to raise the pH of many in vitro procedures prior to phenol extraction. For applications which require an alkaline pH, such as DNA isolation, use phenol buffered to pH ~7.9.

Salt concentration also has an effect on the efficiency of phenol extraction. RNA has traditionally been extracted at high salt concentration (0.2–0.5 M NaCl) to reduce the effect of endogenous ribonucleases. With the use of more effective ribonuclease inhibitors, such as guanidinium salts, optimal RNA yields are obtained in an extraction buffer with low or no salt present.

Chloroform is mixed with phenol to increase the efficiency of nucleic acid extractions. The increased efficiency of extraction is due to chloroform's ability to denature proteins, thus aiding separation of nucleic acid from protein. Phase separation of the extracted solution is also enhanced, thereby assisting in the removal of the aqueous phase with minimal cross contamination from the organic phase. Addition of chloroform to the phenol also aids in the removal of lipids. Often isoamyl alcohol (IAA) is added to the phenol:chloroform to prevent foaming.

Applications:

Notes on Use of Phenol

To measure the pH of phenol: Measurement of phenols with pH paper is not accurate due to breakdown of indicator chemicals on the paper. Mix 2 mL of the organic phase with 8 mL of methanol and 10 mL of water. Measure the pH of the entire sample with a pH meter.

Preparation of crystalline phenol for use is a time-consuming and often hazardous procedure. The Ambion® series of Saturated Phenols eliminates these handling problems.

Product	Catalog #	Applications
Saturated Phenol pH 7.9 ± 0.2	AM9710 – 100 mL AM9712 – 400 mL	Extraction of nucleic acids Add alkaline buffer (included) for pH 7.9
Acid Phenol:Chloroform pH 4.5 ± 0.2	AM9720 – 100 mL AM9722 – 400 mL	RNA isolation Removing DNA from in vitro transcription reactions
Phenol:Chloroform:IAA pH 6.7 ± 0.2	AM9730 – 100 mL AM9732 – 400 mL	Extraction of nucleic acid, when phenol:chloroform is desired. Add alkaline buffer (included) for pH 7.9

References:

1. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K, editors. (1987) *Current Protocols in Molecular Biology*, p 2.1.3–2.1.4, 15.3.8–15.3.11.
2. Wallace DM. (1987) Large- and small-scale phenol extractions. *Methods in Enzymology* **152**: 33–41.
3. Sambrook J, Fritsch EF, and Maniatis T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed.
4. Perry RP, La Torre J, Kelley DE, Greenberg JR. (1972) On the lability of poly(A) sequences during extraction of messenger RNA from polyribosomes. *Biochim. Biophys. Acta* **262**: 220–226.
5. Brawerman G, Mendecki J, Lee SY. (1972) A procedure for the isolation of mammalian messenger ribonucleic acid. *Biochemistry* **11**: 637–641.
6. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**:5294–5299.
7. Chomczynski P and Sacchi N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162**:156–159.

QUALITY CONTROL

Quality Verification: Specifications listed in this document have been verified to pass all criteria.

OTHER INFORMATION

Material Safety Data Sheets: Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion are available 24 hours a day. At www.appliedbiosystems.com, select Support, then MSDS. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest. At www.ambion.com, go to the web catalog page for the product of interest. Select MSDS, then right-click to print or download. Or, e-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com), telephone (650-554-2756; USA), or fax (650-554-2252; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated MSDSs unless you request fax or postal delivery. Requests for postal delivery require 1-2 weeks for processing.

Warranty and Liability: ***For research use only. Not for use in diagnostic procedures.***

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