ProductInformation

PHENOL:CHLOROFORM:ISOAMYL ALCOHOL (25:24:1)
Product Number **P3803**
Storage Temperature 2-8°C

Product Description

Appearance: Clear colorless solution
Component ratio: Phenol:Cholorform:Isoamyl Alcohol (25:24:1)
Phenol phase pH: 6.7 ± 0.2; equilibrated with 10 mM Tris, pH 8.0, 1 mM EDTA

Removal of proteins from nucleic acids can be achieved by extraction with phenol:chloroform solutions. For crude mixtures of nucleic acids, digestion with a broad range proteolytic enzyme prior to the phenol:chloroform extraction may be beneficial. Although proteins are efficiently denatured by phenol, RNase activity is not completely inhibited. Therefore, a small amount of isoamyl alcohol is added to further ensure the deactivation of RNase activity. For DNA extraction, the pH of the phenol phase can be adjusted to 8.0 by equilibration with tris buffer. Sigma offers product P2069 specifically for DNA extractions. This product includes equilibration buffer for the convenience of adjusting the pH.

Disclaimer/Precautions

If phenol solutions come in contact with skin, burns will occur quickly. It is, therefore, required that the user wear appropriate laboratory clothing including gloves and protective eyewear. In case of direct skin contact, rinse extensively with water and seek medical attention if necessary.

Storage/Stability

As supplied, this product is stored at 2-8°C. This product has a shelf life of two years.

Procedure

The protocol for removal of proteins form nucleic acids follows:[1]

1. Mix equal volume of phenol:chloroform:isoamyl alcohol solution with the nucleic acid solution in polypropylene tube with a cap. Mix briefly until an emulsion forms.
2. Centrifuge at 12,000 g for 3-5 minutes at room temperature. The aqueous phase (upper) and organic phase (lower) should be well separated. The interface, which typically appears as an opaque disc, contains the denatured proteins.
3. Transfer the aqueous phase to a new tube. Discard the interface and organic phase.
4. Repeat steps 1-3 until no protein is visible at the interface.
5. (Optional) Mix equal volume of chloroform with the aqueous phase. Mix briefly and centrifuge at 12,000 g for 3-5 minutes. This step will remove any residual phenol.
6. Pipette upper phase into a new tube and precipitate the nucleic acid as desired.

References

1. Sambrook, Fritsch, Maniatis, Molecular Cloning: A Laboratory Manual 2nd Edition, Vol. 3, pages E3 - E4; Cold Spring Harbor Laboratory Press 1989.

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