Phenol extraction of proteins

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Abstract

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Guidelines

Reagents

Liquid (88%) phenol

1% w/w Ficoll 400

0.5M DTT solution (77 mg/ml)

10M LiCl

10% w/v SDS

1% Ponceau S (dye)

Acetone

Purifying proteins

Proteins are easily precipitated from phenol with 10 volumes of methanol. I use Ficol l as a carrier. Ficoll stabilizes the pellet and makes it visible. Four volumes of methanol efficiently precipitates all but the smallest proteins. Acetone precipitates phospholipids. Methanol won't precipitate alcohol soluble proteins (usually of little concern). Methanol precipitates proteins poorly from acidic solutions. An organic base or a buffer (e.g. ammonium acetate) solves this problem. Adding sucrose during protein extraction conveniently 'inverts' phases.

How efficient is phenol extraction of proteins?

The distribution coefficient of proteins into the phenol phase is greater than 100 (Pusztai, A. *Biochem J* 99 , 93 ; 1966, in my experience greater than 1000). Hydrogen bonding to the peptide backbone is important; small peptides likely have lower
distribution coefficients. Phenol is less a denaturant than a solvent. Salt (or higher pH) helps to extract histones and heat, higher pH or denaturants help to strip TMV capsid from the virion. Phenol (above, carbolic acid) was isolated from coal tar in 1834. It served as a bacteriocide in the late 19th century. Phenol extraction first served to purify (deproteinize) carbohydrates (Westphal, et al. *Z Naturforschung B* 7 , 148, 1952). It was subsequently adapted to 'purify' nucleic acids (Kirby, *Biochem J* 64 , 405, 1956). It also separates glycoproteins (Howe, et al, *Meth Enzymology* 28 , 236, 1972) from

erythrocyte membrane non-glycoproteins.

Basic principles
Phenol dissolves proteins (for an interesting example see Cohn, E.J. and Conant, J.B. The molecular weight of proteins in phenol . *PNAS* 12 , 433- 438, 1926) and lipids leaving water soluble matter (carbohydrates, nucleic acids, etc.) in the aqueous layer. Particulate and 'ambiguous' matter remain insoluble. Phenol extraction of nucleoproteins (e.g virus particles) gives pure products. Crude tissue yields complex mixtures, particularly in the aqueous phase.

Preparing phenol

Phenol is often distilled and then saturated with water. Chelators (8-hydroxyquinolin e) are added to inhibit oxidation. Phenol is usually neutralized by dialyzing against a buffer. Biochemists have ignored the acidity of phenol (pK ~10) which is itself the major buffer above pH 7 (and will titrate buffers proportional to the buffer/phenol ratio). It is far simpler to neutralize phenol by adding a weak base (such as tris, I prefer N-ethylmorpholine, a liquid). Adjusting the phenol/tris ratio is more convenient (and reproducible) than dialyzing. Raising pH stimulates oxidation so neutralize phenol immediately before use.

Protocol

Step 1.
To 100 µl of PBCV-1 in a 1.5 conical plastic centrifuge tube add 150 µl of phenol.

Step 2.
Add DTT (10 µl), 10% SDS (20 µl), 10M LiCl (15 µl) and Ponceau S (10 µl).

Step 3.
Heat 5 min at 75°C, mix thoroughly.

DURATION
00:05:00

Step 4.
Allow to cool, centrifuge 1 min (5K).

DURATION
00:01:00

Step 5.
Draw off lower (clear) phase into 1.5 conical plastic centrifuge tube.

Step 6.
Add 1 ml acetone and then 5 µl of 1% Ficoll, mix.

Step 7.
Store 20 min at -20°C.

DURATION
00:20:00

Step 8.
Centrifuge 1 min (6K).

DURATION
00:01:00

Step 9.
Discard supernatant, drain tube, wash ppt once with acetone (ppt sticks to tube) and dry.

Step 10.

Dissolve pellet in 50 µl cracking buffer (1% SDS; 5% glycerol; 0.03125 M Tris-HCl, pH 6.8; bromophenol blue-trace).