# Extraction of deep-Rough LPS (Phenol-Chloroform-Petroleum Ether Extraction Method)

## MATERIALS

- Bacterial cells (Gram negative)
- Phenol redistilled (Sigma: 322111)
- Chloroform (Sigma: 288306)
- Petroleum Ether (40-60 °C) fraction (Sigma: 77379)
- Acetone (Sigma: 270725)
- Diethyl Ether (Sigma: 309969)
- Ice-water bath
- Chemical Hood
- Cold centrifuge
- 100 mL stoppered measuring cylinder

**Preparation of extraction solution:**

- This reaction mixture should be freshly made and stored at <10°C before use.
- Extraction solution is a homogenous mixture of 90% phenol, chloroform and petroleum ether in 2:5:8 v/v ratio.
- Weigh out 9 g of crystalline phenol and dissolve it in 1.1 mL of water to give 10 mL of phenol solution. To this add 25 mL of chloroform and the solution was slowly mixed. Finally add 40 mL of petroleum ether and again mix thoroughly. The solution should be homogenous; if the solution is not homogeneous it can be made homogenous by adding small amounts of crystalline phenol.

**Preparation of the bacterial sample:**

- Bacterial cells are harvested by centrifugation and washed successively with deionized water, 90% ethanol (twice), acetone (twice) and diethyl ether (once). A slow flow of dry nitrogen is given to remove the organics. The dried cells are then grinded finely using a mortar pestle.

## PROCEDURE (Extraction Procedure Should Be Done in a Chemical Hood):

1. Prepare an ice-water bath with a temperature of 10 ± 2°C.

NOTE: Higher temperature will alter the ratio of the extraction solution components and lower temperatures will lead to incomplete LPS extraction.



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2. Weigh out 5 g of finely ground bacterial cells in a 50 mL stoppered round bottom flask (glass).

3. Place the flask on ice-water bath and gradually add 20 mL of the extraction solution with continuous stirring. Add more extraction solution if the mixture is too viscous.

4. Stir the reaction mixture vigorously for 30 min in the ice-water bath maintaining the temperature at10 ± 2°C.

5. Centrifuge the reaction mixture for 10min at 5,000 rpm maintaining the temperature at 10°C.

NOTE: The supernatant should contain the rough LPS and should appear yellow to dark brown in color.

6. Re-suspended the precipitate in the extraction solution and the extraction was repeated once more.

7. Pool the supernatant from the two extractions and remove the organics using a rotor-evaporator.

8. Chloroform and petroleum ether should be evaporated quickly leaving the 90% phenol in the flask.

9. Precipitate the Rough LPS from 90% phenol layer by slowly adding distilled water, 100 µL at a time with shaking the flask slowly. The Rough LPS is completely removed when addition of water results in no further precipitation.

10. Centrifuge at 5,000 rpm for 5 min to remove the Rough LPS from phenol layer. Carefully remove the phenol layer and wash the precipitate using a mixture of 5:1 diethyl ether to acetone mixture.

11. Repeat the washing step at least 4-5 times to completely remove the phenol.

12. Trace amounts of phenol can also be removed by dialysis of the Rough LPS using 1,000 MWCO dialysis tubing (regenerated cellulose)

13. Rough LPS prepared by the PCP method can contain small amounts of membrane phospholipids. These are removed by washing the precipitate with 9:1 aqueous ethanol.


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