LPS extraction (Lab4)

LPS extraction method

1. Phenol water method
2. EDTA
3. Kit extraction

Hot phenol- water method

Protocol

- Bacterial suspension 10^8 cell/ ml is centrifuged 1500 rpm at 5 minutes.
- Wash the pellet twice in PBS
- Suspend the pellet in 5 ml of PBS with sonication 10 min .in ice.
- The pellet should be treated with proteinase K, DNase and RNase **(Why) ?** at 65 C for 1hr.

❖ To prevent nucleic acid contamination

- Equal volume of hot phenol is then added to mixture following by shaking at 65-70 C for15 min.
- Suspension should be cooled on ice .
- Transfer the supernatants to centrifuge tube and phenol phase is then extracted by D.W.
- Add 10 ml of 95% ethanol to the extract and sample should be stored at -20 C overnight in order to precipitate the LPS.

Dialysis: is the separation of practices in a liquid on the basis of differences in their ability to pass through a membrane it can use to eliminated any residual phenol in aqueous phase.

Extraction of LPS by Kit

Protocol

- Harvest the bacteria cell in 30 sec.
- Add lysis buffer for 5min.
- Add chloroform and incubate at 37 C for 10 min.
- Centrifugation and take the supernatant.
- Add purification buffer for 15 min.
- Centrifugation for 5 min, and take pellet.
- Wash the pellet and resolve it.

Note: by kit denature the protein and resolve through the denaturalization in form precipitation, also separated lipid by way of fraction in phenol, extraction easily and quickly.