

SOP: PP025

Production of SDS Soluble Cell Wall Proteins SOP

Materials and Regents:

1. *M. tuberculosis* cell wall (note 1)
2. Phosphate Buffered Saline (note 2)
3. 2% SDS/PBS solution (note 3)
4. Oakridge centrifuge tubes, 50 ml, sterile (4)
5. Platform rocker
6. Cold room, 4°C
7. Sorvall RC5-B centrifuge
8. Sorvall rotor, SS-34
9. Electric pipettor
10. Pipette, 25 ml

Protocol:

1. ____ Transfer cell wall preparation to a sterile 50 ml Oakridge centrifuge tube.
2. ____ Using a 25ml pipette and electric pipettor, add PBS to cell wall (note 5).
3. ____ Cap tube and place on platform rocker in 4°C cold room for one hour.
4. ____ Using Sorvall RC5-B centrifuge and SS-34 rotor, centrifuge cell wall/PBS mixture at 27,000 x g 4°C, for 30 minutes.
5. ____ Discard supernatant and keep pellet.
6. ____ Using a 25ml pipette and electric pipettor , add 2% SDS/PBS solution to pellet (note 5).
7. ____ Cap tube and place on platform rocker at room temperature for two to four hours.
8. ____ Using Sorvall RC5-B centrifuge and SS-34 rotor, centrifuge cell wall/PBS mixture at 27,000 x g, 4°C, for 30 minutes.
9. ____ Using a 25ml pipette and electric pipettor, transfer supernatant equally between three sterile 50 ml Oakridge centrifuge tubes.
- 10 ____ Begin SOP to remove SDS (note 4).

Notes:

1. Material is produced according to SOP PP008.
2. PBS is made according to SOP R001 or is bought-in.
3. 2% SDS/PBS is made as follows: 2 g SDS per 100 ml of PBS.
4. SDS is removed according to SOP SP019.
5. Add at least 30 ml of PBS/ 2% SDS solution in order to have enough volume in Oakridge tube for subsequent centrifugation steps.

Reference:

Hirschfield, G.R., *et al. J. Bacteriol.* **172**:1005, 1990.