SOP: PP025.1
Modified: 6/1/2017 by MCL

Production of SDS Soluble Cell Wall Proteins SOP

Materials and Regents:

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

Protocol:

1._____ Transfer cell wall preparation to a sterile 50 ml Oakridge centrifuge tube.

2._____ Bring the volume to 35 ml by adding PBS to cell wall (note 3).

3._____ Cap tube and place on platform rocker in 4°C cold room for one hour.

4._____ Centrifuge cell wall/PBS mixture at 27,000 x g 4°C, for 30 minutes.

5._____ Discard supernatant and keep pellet.

6._____ Add enough 2% SDS/PBS solution to pellet to bring the volume to 35 ml (note 3).

7._____ Cap tube and place on platform rocker at room temperature for 2-4 hours.

8._____ Centrifuge cell wall/SDS-PBS mixture at 27,000 x g, 4°C, for 30 minutes.

9._____ Transfer supernatant equally between three sterile 50 ml Oakridge centrifuge tubes. Discard the pellet.

10._____ Freeze and lyophilize the material before proceeding to SDS removal (note 4).

Notes:

1. Material is produced according to SOP PP008.
2. 2% SDS/PBS is made as follows: 2 g SDS per 100 ml of PBS.
3. Centrifuge tubes must be full (35 ml) before centrifugation in order to prevent tube collapse.
4. See SOP SP004 for use of the lyophilizer. SDS is removed according to SOP SP019.

Reference:

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