

# Harvesting and Washing

## Aims of the experiment

It is used to obtain Bacteria from the cultivation and prepare cell pellets for the cell extract production. It is proceed according Sun, Z. Z et al., 2013.

## Materials

- 2.5L sterile shaking flasks
- 1L sterile centrifuge bottles with grey cap
- (250ml sterile centrifuge bottles with grey cap)
- Sterile 50ml falcon tubes

## Harvesting Procedure

1. Transfer cultures of OD 1.8 – 2.0 from the Cultivation Protocol to large centrifuge bottles, centrifuge at max speed or at 5000g for 15 min at 4°C.

## Washing Procedure

Keep the cells always on ice!

1. Remove supernatant by decanting the bottle and blotting it onto paper.
2. Add 200ml S30A buffer to each of the centrifuge bottle and resuspend the pellet by shaking (DON'T vortex cells, alternate between shaking bottles and cooling cells on ice until the pellets have been completely resuspended).
3. Centrifuge at max speed or at 5,000g for 15 minutes at 4°C.
4. Repeat step 2 and 3.
5. Remove supernatant, add 40ml S30A buffer to each of the bottle and resuspend pellets.
6. Transfer cell suspension to 2 (or more) pre-chilled and weighed 50ml Falcon tubes.
7. Centrifuge the Falcon tubes at 2,000g for 8 minutes at 4°C, remove supernatant by decanting.
8. Centrifuge Falcon tubes at 2,000g again for 2 minutes at 4°C, remove residual supernatant by pipetting.
9. Record the mass of cell pellet.

10. After cell washing, flash freeze the pellets in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ , or keep on ice to immediate process on sonication.
11. Continue with Sonication Protocol or Bead Beating Protocol depends on the desired lysis method.