

Cell Cultivation

Shaking Flask method

Aims of the experiment

Cultivation is used for preparing enough bacteria for cell extract production. The cultivation is proceed according Sun, Z. Z et al., 2013.

Materials

- 2.5L sterile shaking flasks
- 1-L sterile centrifuge bottles with grey cap
- (250ml sterile centrifuge bottles with grey cap)
- Sterile 50ml falcon tubes
- 2YT+P media
- Rosetta strain
- Chloramphenicol

Preparation

1. Prepare desired amount of 2YT+P media for cultivation: 660 mL Media per 2.5L shaking flask (refer to Buffer and Media Preparation Protocol).
2. Prepare required amount of sterile materials in advance to facilitate the process of cultivation.
3. Prepare an Overnight Culture of Rosetta in 2YT+P of 1:100 culture dilution, addition with 1:1000 Chloramphenicol (Cm).

Procedure

1. Prepare required shaking flasks with 660 mL of 2YT+P each using a scale, addition with 1:1000 Cm.
2. Inoculate Cultures with a 1:100 dilution of the overnight culture.
3. Incubate Cultures at 37°C at 250 rpm, check OD600 periodically.
4. Harvest cells at OD 1.8-2.0, cool the harvested cultures immediately on ice once the target OD has been reached.
5. Continue with the Harvesting and Washing Protocol.