

## 1. LB medium preparation

### *Material*

LB Broth Agar Powder, Miller  
LB Broth Powder, Miller  
Ultrapure (UP) Water  
Double Distillation Water (ddH<sub>2</sub>O)  
Ampicillin

### *Step*

#### Making LB liquid medium

- ① Weigh 12.5g of LB Broth Powder.
- ② Add the powder into 500ml UP Water.
- ③ Autoclave entire bottle of LB medium under 121 Celsius degree for 20 minutes.

#### Making LB solid medium

- ① Weigh 12g of LB Broth Agar Powder.
- ② Add the powder into 300ml UP Water.
- ③ Autoclave entire bottle of LB medium under 121 Celsius degree for 20 minutes.
- ④ Pour the medium into culture dishes in the ultraclean worktable.

#### Making LB selective medium

- ① Weigh 12g of LB Broth Agar Powder.
- ② Add the powder into 300ml UP Water.
- ③ Autoclave entire bottle of LB medium under 121 Celsius degree for 20 minutes.
- ④ Weigh 0.98g of Ampicillin.
- ⑤ Add the ampicillin into 9.8ml ddH<sub>2</sub>O. The ddH<sub>2</sub>O is sterilized previously. Shake the mixture fully.
- ⑥ Filter the mixture through a filter to remove microorganism.
- ⑦ Add 1μl of mixture into an EP tube with 999μl ddH<sub>2</sub>O. The ddH<sub>2</sub>O is sterilized previously.
- ⑧ When the LB medium cool down to nearly 50°C, add 300μl antibiotic mixture into it in the ultraclean worktable, then pour it into culture dishes.