

## Chemically Competent Cells Preparation

### Protocol for *E. coli* DH5 $\alpha$ Chemically Competent Cells Preparation

#### Materials:

- 1 LB plate
- 2 50mL Erlenmeyers Flask
- 1 125mL Erlenmeyer Flask
- LB Broth (100mL aprox.)
- 2 50mL Conical Centrifuge Tubes 50mL
- Ice and cooler
- CaCl<sub>2</sub> 0.1M sterilized
- CaCl<sub>2</sub> 0.1M + 15% Glycerol sterilized
- Microcentrifuge Tubes 1.5mL (previously chilled)

#### Procedure:

##### **Day 1**

1. Streak out a colony of *E. coli* DH5 $\alpha$  on a LB plate without antibiotics. Incubate at 37°C for 24 hours.

##### **Day 2**

1. Pick a colony from the fresh LB plate culture and inoculate 20mL of LB media. Incubate overnight at 37°C and 180rpm.

##### **Day 3**

1. Inoculate 10mL starter culture of LB media with 100  $\mu$ L of the previous day culture in a 25mL Erlenmeyer flask (media should be preheated to aprox. 37°C). Incubate at 37°C and 180rpm until it reaches an OD<sub>600</sub> between 0.3 and 0.5 (it takes around 3 hours to reach the required OD<sub>600</sub>).

2. Inoculate 45mL of LB media with 5mL of the starter culture. Incubate at 37°C and 180rpm until it reaches an OD<sub>600</sub> between 0.4 and 0.55 (it takes around 2 hours to reach the required OD600).
3. Distribute the volume in two 50mL conical centrifuge tubes.

Note: Cold chain must be maintained in all the following steps.

4. Centrifuge tubes at 2700g and 4°C for 10 minutes (the centrifuge should be previously chilled).
5. Discard supernatant and resuspend pellets in 1mL of pre chilled CaCl<sub>2</sub> 0.1M. Transfer all the cells to the same conical centrifuge tube and take it to a final volume of 25mL with CaCl<sub>2</sub> 0.1M. Incubate overnight at 4°C.

#### **Day 4**

Note: Cold chain must be maintained in all the following steps.

1. Centrifuge at 2700g and 4°C for 10 minutes (the centrifuge should be previously chilled).
2. Discard supernatant and resuspend pellets in 2mL of pre chilled CaCl<sub>2</sub> 0.1M + 15% glycerol.
3. Aliquot 50µL in pre chilled 1.5mL centrifuge tubes and use immediately or store at -20°C.