Protocol for Protein induction in *Escherichia coli* BL21 strain.
Protocol Code: InEc

Materials

- LB medium
- Ampicillin
- Spectophotometer
- Spectophotometer cuvettes
- Flask
- IPTG
- Erlenmeyers
- Laminar Flow Hood
- Shaker
- Centrifuge

Procedure

Day 1.

1. Preculture

Prepare a preculture with LB liquid medium, 100 ug/mL of ampicillin and one colony of the desire bacteria. Incubate O/N at 37°C.

Day 2.

1. Culture

-Prepare a culture with fresh LB medium, 100 ug/mL of ampicillin and 10% of preculture. Before inoculation, take 1 mL of the sample in a spectrophotometer cuvette and tag as “Blank sample”.
-Grow 2½ hours at 37 °C in a shaker.

3. Prepare IPTG

-Prewarm 1 M IPTG to 45°C.

4. Induction

-When the OD is between 0.5 and 0.6 centrifuge 1 mL for 1 min at 4000 rpm room temperature and place the supernatant and pellet in different tubes. Label them as “supernant before induce SDS” and “pellet before induce SDS”. Freeze it at -20°C until needed.

-Add 1 mM IPTG and incubate the culture overnight in a shaker.

5. Final centrifugation

-After 2 hours of incubation, centrifuge 2 mL of the sample for 1 min at 4000 rpm and 4°C. Store the supernatant and pellet in different tubes. Label them as “supernatant after induce SDS” and “pellet after induce SDS”. Freeze at -20°C until needed.
-Centrifuge the culture for 30 min at 5500 g and 4°C. Place the supernatant and pellet in different tubes. Label as “supernatant after induce” and “pellet after induce”.
5.3. Centrifuge the “supernatant after induce” again with same conditions to ensure the absence of cells. Freeze at -20°C until needed.

Notes

The lysin is incubated after induction at 21°C, and the AIP at 37°C.

References

Dunne, M., Mertens, H. D. T., Garefalaki, V., Jeffries, C.M. et al. (2014). The CD27L and CTP1L Endolysins Targeting Clostridia Contain a Built-in Trigger and Release Factor. PLoS Pathog 10(7): e1004228. doi:10.1371/journal.ppat.1004228