

**Title of Procedure:** Induction Protocol - To induce protein expression after cell culturing.

**Materials/Reagents:**

- Overnight transformed cells in the petri dish.
- Ampicillin.
- 2xyt

**Protocol:**

- 1.) Inoculate single colonies from transformation into a starter culture of LB media containing 100 ug/ml ampicillin and grow it up at 37°C **overnight**.
- 2.) Inoculate the starter culture at a 100 - fold dilution into a 2xyt medium containing 100 µg/mL ampicillin.
- 3.) Grow up the diluted medium at 37°C until the optical density measured at 600 nM (OD600) reaches 0.5-0.7 (**~4 Hours**).
- 4.) Take a 1 mL sample immediately before induction (noninduced control). Pellet this 1mL tube and then resuspend in 50 uL 1x SDS-PAGE sample buffer. Freeze sample at -20 degrees until needed for SDS-Page.
- 5.) Induce protein expression by adding IPTG to a final concentration of ??? mM for ??? hours or overnight at a temperature of ??? degrees.

16 degrees, 0, 100 micromolar, 200, 500, 1000, overnight - UT Austin.

Make IPTG freshly

Secretion to get proteins out?

For purification, use 400 mL

**References:**

<https://2019.igem.org/Team:Toronto/Experiments>