# AUGUST

WEEK 4

24/08/21

# Aim: **Check whether proteins are expressing in inclusion bodies or not**

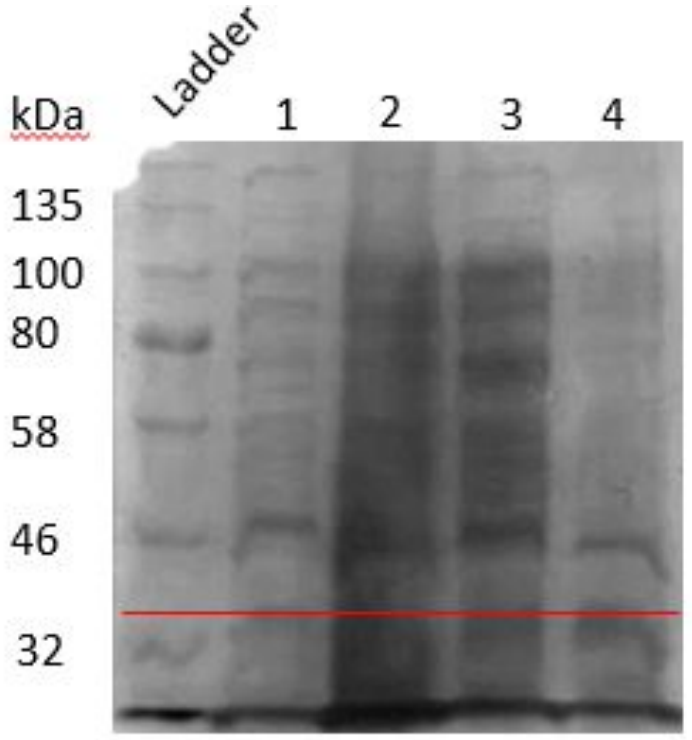
Protocol:

- Primary inoculum- 10 ml lb + kan(50ug/mL). Incubate for 12hr
- Secondary inoculum- 1050 ul primary inoculum 105 ml lb+ kan (50ug/mL). Incubate till 0D reaches 0.6. (preserve 1ml of secondary inoculum as a control)
- Add 1mM IPTG(200ul of 0.5M IPTG) and incubate at 16℃ for 12hr.
- Pellet it down. Take some pellet and add loading dye buffer.
- Take some pellet and add lysis buffer.
- Sonicate it and then centrifuge at 4C for 11000 rpm for 45 mins..
- Dissolve both pellet and supernatant in loading buffer separately.
- Perform SDS [Stacking Gel (5%), Resolving Gel (10%)]

# SDS Page

Annotations :

1:Sample from Supernatant (After sonication)
2:Sample from Pellet (After Sonication)
3:Sample from Pellet (After Sonication)
4:Sample from Supernatant (After sonication)




Result: The protein is coming in both supernatant and pellet at 16°C

# Protein Extraction

1) Inoculation
2) Induction
3) Protein Purification

# Inoculation and Induction

- Primary inoculum incubated overnight (10mL) (13hrs).
- Secondary inoculum (1L) incubated till OD600 reached 0.6
- Secondary inoculum induced with 1mM IPTG and incubated overnight at 16 C.
- Cells pelleted down at 13,000 RPM for 15 mins and stored at -20 C.

# Lysis and Sonication

27/08/2021

Lysis Buffer

| Components | Stock concentration | Working Concentration | Volume required for 50 mL solution |
|---|---|---|---|
| Sodium Phosphate Buffer (pH 7) | 0.1 M | 50 mM | 10 mL |
| PMSF | 1 M | 1 mM | 20 uL |
| Sodium Chloride | 2 M | 0.5 M | 5 mL |
| BME | | 0.05 % of lysis buffer | 10 uL |
| Glycerol | | 5 % of lysis buffer | 1 mL |
| Triton X-100 | 100 % | 0.5 % of lysis buffer | 100 uL |
| Protease Inhibitor | 1 tablet(EDTA free) | 1 tablet (EDTA free) | 1 tablet (EDTA free) |
| Autoclaved Milli Q | To make up 50 mL | | 3.87 mL |

28/08/2021

# Sonication

| | |
|---|---|
| On time | 30 secs |
| Off time | 30 secs |
| Amplitude | 55% |
| Total cycles | 15 |
| Total time | 15 mins |

Sonication carried out in pulse mode

# Protein Purification (Ni-NTA)

31/08/2021

Equilibration Buffer

| Component | Stock Concentration | Working Concentration | Volume for 50 mL |
|---|---|---|---|
| Sodium Phosphate Buffer (pH 7) | 0.1 M | 50 mM | 10 mL |
| PMSF | 1 M | 1 mM | 20 uL |
| Sodium Chloride | 2 M | 0.5 M | 5 mL |
| BME | | 0.05 % | 10 uL |
| Glycerol | | 5 % | 1 mL |
| Triton X-100 | 100 % | 0.5 % | 100 uL |
| Autoclaved Milli Q | Make upto 50 mL | | 3.87 mL |

Wash Buffer (50 mL)

| Component | Stock Concentration | Working Concentration | Volume for 50 mL Wash Buffer |
|---|---|---|---|
| Tris Base (pH 8) | 1 M | 20 mM | 1 mL |
| Sodium Chloride | 5 M | 400 mM | 4 mL |
| EDTA | 0.5 M | 1 mM | 100 uL |
| Imidazole | 1 M | 25 mM | 1.25 mL |
| Autoclaved MilliQ | Make upto 50 mL | | 43.65 mL |

## Elution Buffer (50 mL)

| Component | Stock Concentration | Working Concentration | Volume for 50 mL Elution Buffer |
|---|---|---|---|
| Tris Base (pH 8) | 1 M | 20 mM | 1 mL |
| Sodium Chloride | 5 M | 150 mM | 1.5 mL |
| EDTA | 0.5 M | 1 mM | 100 uL |
| Imidazole | 1 M | 250 mM | 12.5 mL |
| Autoclaved MilliQ | Make upto 50 mL | | 34.9 mL |

# Ni-NTA Purification

- The flow through (filtrate collected after loading solubilised protein) and wash buffer were collected separately in 50 mL falcon tube.
- The elution flow through was collected in fractions in 20, 2 mL Eppendorf tubes.