SDS-PAGE

This protocol describes the separation of proteins by denaturing polyacrylamide gel electrophoresis, followed by Coomassie staining.

Materials

- 4X Laemmli buffer
- DTT
- 1.5 mL Eppendorf tubes
- 4–20% Tris-glycine pre-cast gel (Invitrogen)
- Protein ladder
- Tris-glycine-SDS running buffer (Fisher BioReagents)
- Rapid protein stain, Coomassie blue (Westburg Life Sciences)
- Water

Equipment

- Thermomixer (Eppendorf)
- Microcentrifuge (Eppendorf centrifuge 5424)
- Electrophoresis unit (Bio-Rad)
- See-saw rocking shaker (Labnet)
- Gel imager
- Large petri dish
- Nitrile gloves
- Pipettes and pipette tips (2–20, 20–200 µL)

Protocol

1. In a clean 1.5 mL Eppendorf tube, mix 15 µL of your sample with 5 µL of DTT and 4X Laemmli buffer mixed in a 1:10 proportion.
2. Incubate at 95 ℃ for 10 min.
3. Centrifuge the samples briefly.
4. Load the samples into the wells, and 6 µL of protein ladder into the first well.
5. Run the gel in Tris-glycine-SDS running buffer at 200 V for 30–35 min.
6. At the end of the run, open the cassette and transfer the gel to a large clean petri dish. Keep the lid on as much as possible to prevent keratin contamination.
7. Stain with the rapid protein stain for ∼20 min on the see-saw rocking shaker.
8. De-stain with water for 20 min, then image the gel.

Protein Expression

This protocol describes the expression of a protein of interest in *E. coli* BL21 (DE3) from a 1 L culture. It is followed by the protein isolation and purification protocol.

Materials

- *Escherichia coli* BL21 (DE3) transformed with the plasmid of interest
- 1 L LB medium
- Antibiotic of interest (here kanamycin, 50 mg/mL)
- IPTG (1 M)

Equipment

- 5 L Erlenmeyer flask
- Shaking incubator (37 °C and 20 °C)
- Spectrophotometer (OD_{600})
- Ice and ice bucket
- Pipettes and pipette tips (10 µL–5 mL)

Protocol

1. Sterilise the 5 L Erlenmeyer flask and prepare 1 L of LB medium.
2. Inoculate 10 mL of LB medium containing the appropriate concentration of antibiotic from a glycerol stock of the transformed BL21 (DE3). Grow overnight.
3. The next day, add the 10 mL culture to the 1 L of LB medium containing the appropriate amount of antibiotic.
4. Grow at 37 °C and 180 rpm until the culture reaches an OD_{600} between 0.4 and 0.6.
5. Arrest growth by placing the flask on ice for 1 h.
6. Take a 1 mL sample and measure the OD_{600}. Calculate the volume of that sample corresponding to $OD_{600} = 0.15$ and keep it for SDS-PAGE.
7. After the cold shock, add IPTG to a final concentration of 1 mM.*
8. Incubate the culture overnight at 20 °C and 180 rpm.
9. The next day, take a second 1 mL sample, measure the OD_{600}, and again keep the volume corresponding to $OD_{600} = 0.15$ for SDS-PAGE.

* Lower concentrations work almost as well; 1 mM is used here to maximise yield

Protein Isolation and Purification

This protocol describes the purification of a few milligrams of a His-tagged protein of interest from a 1 L culture, by Ni-NTA affinity chromatography.

Materials

- *E. coli* BL21 (DE3) culture from the day before
- Wash buffer (500 mM NaCl, 20 mM Tris-HCl, 20 mM imidazole, pH 8)
- Elution buffer (500 mM NaCl, 20 mM Tris-HCl, 250 mM imidazole, pH 8)
- Storage buffer, e.g. HBS (140 mM NaCl, 25 mM HEPES, 0.37 mM K_2HPO_4, pH 7.4)
- cOmplete EDTA-free protease inhibitor (1 tablet per 50 mL)
- Ni-NTA agarose
- Glycerol (60%)

Equipment

- Cold centrifuge (4 °C)
- Sonicator
- Affinity columns
- 50 mL Greiner tubes
- 20 mL syringe and 0.45 µm syringe filter
- Centrifugal ultrafilter
- 10 mL pipettes
- Ice and ice bucket

All steps are performed at 4 °C or on ice, and the column must never run dry.

Protocol

1. Pellet the culture by centrifugation at 6000 × g for 15 min at 4 °C.
2. Discard the supernatant and resuspend the pellet in wash buffer with protease inhibitors (∼30 mL per 1 L culture pellet).
3. Sonicate for 15 min at 40% amplitude, 1 s pulses with 2 s rests. Take an aliquot for SDS-PAGE.
4. Centrifuge for 30 min at 30 000 × g at 4 °C to separate the soluble and insoluble fractions. Take an aliquot of each for SDS-PAGE.
5. Filter the supernatant through a 0.45 µm syringe filter into a new 50 mL Greiner tube.
6. Add Ni-NTA 1:5 (Ni-NTA:fraction) and incubate for 30 min at 4 °C while rotating.
7. Add the mixture to an affinity column and let the resin settle completely by gravity (15 min).
8. Break the snap-off cap at the bottom of the column and collect the flowthrough. Take an aliquot for SDS-PAGE.
9. Wash the column with 20–30 column volumes of wash buffer (60–90 mL for a 3 mL Ni column), pipetting slowly against the wall so the bed is not disturbed. Take an aliquot for SDS-PAGE.
10. Elute the protein with 4–5 mL of elution buffer and collect the fraction. Take an aliquot for SDS-PAGE.
11. Exchange the buffer into the storage buffer of choice using a centrifugal ultrafilter. Take an aliquot for SDS-PAGE.
12. Add 60% glycerol to a final concentration of 5%, then store in aliquots at −20 °C or −80 °C.