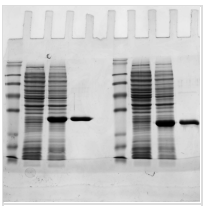

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SDS-PAGE

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SDS-PAGE gels are used to visualize proteins. This protocol describes how to prepare all the buffers required for casting and running SDS-PAGE gels, as well as how to prepare whole cell samples.

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Buffers 10m

10m

1 4X Resolving Buffer (1.5 M Tris-HCl, pH 8.8)

- Add 90.75 g Tris **P212121** to **400 mL** DI water
- Titrate the solution with ~18% HCl to pH 8.8
- Add water to a final volume of **500 mL**
- Store at 4°C

2 4X Stacking Buffer (0.5 M Tris-HCl, pH 6.8) 10m

- Add 30.25 g Tris **P212121** to **400 mL** DI water
- Titrate the solution with ~18% HCl to pH 6.8
- Add water to a final volume of **500 mL** DI water
- Store at 4°C

3 10X Running Buffer 15m

- Weigh 30 g Tris Tris **P212121** .
- Weigh 144 g Glycine **Contributed by users** .
- Weigh 10 g Sodium Dodecyl Sulfate **P212121** SDS.
- Dissolve in **1000 mL** water.
- pH should read 8.3. No pH adjustments are needed.
- Dilute to 1X before use.
- Store at room temperature.

4 10% Ammonium Persulfate (w/v) 5m

- Add 1g of Ammonium persulfate **Contributed by users Catalog #A3678** to **10 mL** of DI water
- Store at 4°C

5 10% SDS (w/v) 5m

- Add 10g of Sodium Dodecyl Sulfate **P212121** to **100 mL** DI water
- Store at room temperature

6 3X Laemmli Buffer 10m

- 2.4 mL 1 M Tris pH 6.8
- 3 mL 20% SDS
- 3 mL glycerol
- 1.6 mL beta mercaptoethanol
- a drop of bromophoenol blue

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Gel Casting 5m

7 In an Eppendorf tube combine 5m

- **0.5 mL** 30% Acrylamide: Bisacrylamide (29:1)
- **0.5 mL** DI water
- **10 µL** APS
- **1 µL** TEMED

Pipette **200 µL** down the right side, and **200 µL** down the left side

Allow to solidify for **00:05:00**

8 Resolving Layer

This following recipe makes a 12% SDS-PAGE. For optimal resolution of large proteins (25-200 kDa), you should use smaller concentration of acrylamide (8%), and for resolution of small proteins (4-70 kDa), you should use higher percentage of acrylamide (12-15%). You can calculate a recipe for a different gel percentage using https://www.cytographica.com/lab/acryl2.html

8.1 Combine

- **1.645 mL** DI water
- **1.645 mL** 30% Acrylamide: Bisacrylamide (29:1)
- **1.25 mL** 4X Resolving Buffer (1.5 M Tris, pH 8.8)
- **50 µL** 10% SDS
- **50 µL** 10% APS
- **5 µL** 4X Resol Buffer (1.5 M Tris, pH 8.8)

TEMED must be used in a fume hood

Add APS just before casting as the gel begins to polymerize immediately after addition of APS.

8.2 20m

- Pour into the mold, leaving ~2 cm below where the bottom of the comb will be
- Cover with a layer of isopropyl alcohol (IPA)
- Wait **00:20:00** for gel to solidify

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9 Stacking Layer

The stacking layer helps all the proteins get lined up so all proteins enter the resolving layer at the same time

9.1 Dump out any excess IPA

10m

Combine

- **2.6 mL** DI water
- **1 mL** 30% Acrylamide: Bisacrylamide (29:1)
- **1.25 mL** Stacking Buffer (0.5 M Tris, pH 6.8)
- **50 µL** 10% SDS
- **50 µL** 10% APS
- **5 µL** TEMED

TEMED must be used in the fume hood
Add APS just before casting as the gel begins to polymerize immediately after addition of APS.

9.2

15m

- Pour into the mold
- Place the comb
- Wait **00:15:00** for gel to solidify
- Move to water storage

Sample Preparation

10 Preparation of Whole Cell Samples for SDS-PAGE analysis

Collect whole cell samples before and after induction, and normalize to the cell mass. This allows you to compare the protein expression before and after inducing cells.

10.1

5m

- Heat the waterbath or heatblock to **95 °C**
- Read the optical density at 600 nm (OD600) of the cell culture using a photometer.

Note: the OD600 reading should be within the linear range of the photometer. If the reading appear to be lower than 0.1 or higher than 0.9 concentrate or

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*dilute the cell sample accordingly in order to ensure the OD reading to fall within the 0.1- 0.9 range. If you dilute or concentrate the sample remember to calculate back the initial concentration (e.g. if you diluted 5 times the initial concentration is y*5, where y is the reading you have obtained from the diluted concentration)*

10.2 10m

- Calculate the amount of culture to harvest to have a cell pellet equivalent to 1mL of OD600 =1.

Calculate the amount of biomass using the formula

$C_i * V_i = C_f * V_f$

where C stands for "concentration", V stands for "volume", i stands for "initial" and f stands for "final". Therefore:

y OD600 * x mL = 1 OD600* 1 mL

where y is the OD600 reading and x is the volume to be calculated. Therefore

x mL = (1 * 1)/y

- Transfer the amount of culture as calculated into an appropriate tube and centrifuge it at 7,000 rpm for **00:10:00** in tabletop centrifuge
- Discard the supernatant

10.3 11m

- Transfer **150 µL** of 1X Laemmli Sample Buffer in the tube and resuspend the pellet by pipetting.
- Transfer the tube to the water bath/heatblock, inserting it into the floaters and incubate the tube at **95 °C** for **00:05:00**
- Transfer the tube on ice for **00:01:00**
- Transfer the tube in a bench-top centrifuge and centrifuge at room temperature at max speed (e.g. 13,000 xg) for **00:05:00**
- Transfer the supernatant to a final 1.5 microcentrifuge tube. *Note: the pellet won't be visible. Remove the supernatant without touching the bottom of the tube with the pipette tips to avoid carry-over of membranes. This step is necessary to remove membranes and debris that will affect a good quality run of the samples on the SDS-PAGE gel.*
- Load **10 µL** of the supernatant on the SDS-PAGE gel.
- Store the remaining sample at **-20 °C** .

11 Purified Protein 5m

- Add **2.5 µL** of 3X Laemmli Buffer to **7.5 µL** of sample

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- Incubate **00:05:00** at **95 °C** .
- Load the sample onto the SDS-PAGE gel.

Running the Gel 1h 30m

12 1h 30m

- Place in gel running box and cover with running buffer
- If running only one gel, make sure the other side has a dummy gel cassette inserted.
- Fill the space between the two gels with 1X running buffer.
- Add **7.5 µL** ladder with dye to a well. Add 7.5 - 10 uL samples to wells.
- Run at 80V until the loading dye reaches the resolving/stacking layer interface.
- Run at 180V until the loading dye reaches the end of the gel.
- Remove gel from casing. Place in petri dish and cover with Coomassie blue dye. Allow to stain overnight.

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