Protocol: SDS-PAGE for analysis of proteins

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Reviewed by: Rytis

Buffer & Solution Recipes

Notes: You don't need to prepare any buffer by yourself. Here is just the information for buffers.

1. 2× SDS Loading Buffer (30 mL)

Ingredients:

- 3.75 mL 0.5 M Tris-HCl (pH 6.8)
- 15.0 mL 50% Glycerol
- 6.0 mL 10% SDS
- 0.3 mL 1% Bromophenol Blue
- Distilled H_2O to 30 mL
- Add β-mercaptoethanol fresh before use (e.g. 5% final: 50 µL per 950 µL)

Instructions:

1. Dissolve all reagents in a tube.
2. Adjust volume to 30 mL with distilled H_2O.
3. Store at 4°C.

2.10× SDS-PAGE Running Buffer (pH ~8.3, 1 L)

Ingredients:

- 30.3 g Tris base
- 144.1 g glycine
- 10.0 g SDS
- Distilled H_2O to 1 L

Instructions:

1. Dissolve all ingredients in ~800 mL distilled H_2O.
2. Adjust volume to 1 L with distilled H_2O.
3. Store at room temperature.
4. Dilute 1:10 to make 1× running buffer before use.

Final concentrations of 1× running buffer:

- 25 mM Tris
- 192 mM Glycine
- 0.1% SDS
- pH ~8.3

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3. Coomassie Staining Solution (100 mL)

Option 1: Standard stain (high background, strong signal)

Ingredients:

- 50% methanol (50 mL)
- 10% acetic acid (10 mL)
- 0.1% (w/v) Coomassie Brilliant Blue R-250 (0.1 g)
- Distilled H_2O to 100 mL

Instructions:

1. Dissolve dye in methanol and acetic acid.
2. Add distilled H_2O to 100 mL.
3. Store at room temperature.

Option 2: High-sensitivity, lower background

Ingredients:

- 1% methanol (1 mL)
- 10% acetic acid (10 mL)
- 0.2% (w/v) Coomassie Brilliant Blue R-250 (0.2 g)
- Distilled H_2O to 100 mL

Instructions:

4. Dissolve dye in methanol and acetic acid.
5. Add distilled H_2O to 100 mL.
6. Store at room temperature.

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4. Destaining Solution (100 mL)

Option 1: Standard destaining

Ingredients:

- 50% methanol (50 mL)
- 10% acetic acid (10 mL)
- Distilled H_2O to 100 mL

Instructions:

1. Mix all ingredients.
2. Store at room temperature.

Option 2: Gentle destaining

Ingredients:

- 10% methanol (10 mL)
- 10% acetic acid (10 mL)
- Distilled H_2O to 100 mL

Instructions:

3. Mix all ingredients.
4. Store at room temperature.

Instructions

Taking cultivation samples for SDS-PAGE

1. Take two samples from the culture for sampling points, one for measuring OD and one for SDS-PAGE, in order to normalize the cell concentration in SDS-PAGE samples.
2. Prepare disposable cuvettes with water for suitable dilution of the cultures. Ensure that the blank is also diluted the same way as the samples. They should be diluted enough to maintain absorbance below 0.3. Prepare small pieces of parafilm for mixing of cuvette content before measuring.
3. Prepare labeled 1.5 mL tubes for SDS-PAGE sample.
4. Take out the culture from the incubator and mix before taking the samples. Take a sample for OD measurement from the culture liquid, and transfer it directly to the cuvette with water. Make sure the outside of the pipette tip is free from culture droplets before transferring it to the cuvette.
5. Take another sample from the culture and transfer it to the Eppendorf-tube. For most part of the cultivation (OD below ~10) a 500 ul will work well. For higher ODs, 250 ul will be sufficient.
6. Put the culture back in the incubator if the cells should be cultivated any further.
7. Place a piece of parafilm over the cuvette and mix the content by inverting the cuvette a few times. Then, measure OD_{600} in the cuvette. Calculate the OD in the culture.
8. Centrifuge the eppendorf tube at 8000 rpm for 1 minute in the microcentrifuge. Carefully remove and discard the supernatant.
9. Using the culture OD, calculate the volume of SDS-PAGE loading dye needed to obtain OD=10. (use the formula $c1 \times v1 = c2 \times v2$).

OD culture * volume of culture taken = 10 * volume of loading dye

10. Use a pipette to suspend the cell pellet in the appropriate volume of SDS-PAGE loading dye to give OD=10.

SDS-PAGE Sample Preparation

Materials:

- 2× SDS Loading Buffer (prepared fresh or thawed)
- Heat block (95–100 °C)
- Precast SDS-PAGE gels
- 1× SDS Running Buffer
- Protein ladder

Protocol:

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11. Heat samples at 95 °C for 10 min. Use the safe cap to prevent the lids from popping out. If the safe cap is not available, poke a hole on the top of the lid of the centrifuge tube.
12. Store the samples at 4°C for a few days, or -20°C for longer storage.
13. While samples incubate, assemble precast gel in the electrophoresis chamber.
14. **The precast gels have a tape on the bottom which needs to be removed**. This is important for the electrophoresis to work.
15. Put the precast gel into the cassette. If you only run one gel, put another plastic plate in the cassette.
16. Fill chamber with 1× SDS Running Buffer; Check for leaks. If you only run one gel, the chamber should still be filled up to reach the “2 gels” level.
17. To remove the comb, position your thumb on the middle of the comb and pull it upward in one smooth motion, slowly and carefully.
18. Use the equipment with sharp ends (like nails) if you only run one gel. Otherwise, use the other one.
19. Ensure that the cassette is facing toward you before loading the samples:
    - 5–10 µL soluble protein sample
    - 5–10 µL total protein sample
    - 5 µL protein ladder
20. Carefully pour 1× SDS Running Buffer into the cassette until it overflows.
21. Run electrophoresis:
    - 80 V for ~10–15 min until samples enter separating gel
    - Check if there is any bubble, if not, check if the cassette is the correct one.
    - Increase to 160 V and run until dye front reaches bottom (~30–40 min)

Post-Run Gel Processing

Staining and Destaining Solutions:

- Prepare staining solution fresh (e.g., 20 mL per gel).
- Prepare destaining solution fresh (enough to cover gel).

Protocol:

1. Carefully transfer the gel from the cassette to a plastic box with distilled water using the green tool or a scalpel. Remove and discard the stacking gel (top part). Wash the separating gel gently and discard the water. **If this is your first time doing this, please ask for assistance**.
2. **In a fume hood**, add staining solution to the gel and gently shake for ~1 hour until bands are visible. Discard staining solution in appropriate waste container.
3. **In a fume hood**, cover the gel completely with a destaining solution and gently shake for 10 minutes. Discard destaining solution in appropriate waste container.
4. Repeat the destaining step for 30 minutes and then discard the solution.
5. Repeat the destaining step for 1 hour or until gel background is clear and bands are sharp.
6. Analyze gel using GelDock.
    - Use a white background, not the plastic cutting screen.
    - Use Epi white, not Trans UV.

Expected size of proteins

Enzyme	***Size (Da)***
ci2350-His	70.57
ci2350-MBP	70.57 + 40.3 = 110.89
ci2350-TrxA	70.57 + 11.8 = 82.37
ci2350-SUMO	70.57 + 11.3 = 81.9
AKR1D1-His	38.41
AKR1D1-MBP	38.41 + 40.3 = 78.71
AKR1D1-TrxA	38.41 + 11.8 = 50.21
AKR1D1-SUMO	38.41 + 11.3 = 49.71
atcBCA	**31.65** + **17.64** + **87.21** = **135.41**

moeABCDE_mobAB	37.3 + 18.7+17.5 + 8.8 + 17 = 99.3 29.6 + 19.4 = 49

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