SDS-PAGE Protocol

Purpose

SDS-PAGE (Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis) is used to separate proteins based on molecular weight. SDS denatures proteins and imparts a uniform negative charge, eliminating differences in native charge and structure. Proteins are then resolved according to size through the sieving effect of the polyacrylamide gel.

Materials and Reagents

- **Equipment**

Electrophoresis tank, power supply, and Shaker

- **Gels**

12% resolving gel and **4%** stacking gel

- **Buffers and Reagents**

PAGE Staining Solution (1L): Prepared by dissolving **1g** Coomassie Brilliant Blue R250 in **450mL** methanol, **450mL** deionized water (ddH_2O), and **100mL** acetic acid.

PAGE Destaining Solution (1L): Prepared from **450mL** methanol, **450mL** ddH_2O, and **100mL** acetic acid.

5×Loading Buffer (5mL): Prepared by mixing **1.25mL** 1M Tris-HCl (pH6.8), **0.5g** SDS, **25mg** bromophenol blue, **2.5mL** glycerol, and **1.25mL** ddH_2O, followed by addition of **250μL** β-mercaptoethanol.

10×Running Buffer (1 L) : Prepared by dissolving **30.2g** Tris, **188g** glycine, and **10g** SDS in ddH_2O to a final volume of **1L**. **Dilute to 1×with ddH_2O before use in electrophoresis**.

- **Samples and Standards**

Protein samples (ECD proteins, **membrane proteins**, antibodies), and Protein marker (such as Thermo PageRuler™ Plus, Cat. No. 26619)

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Experimental Steps

1. Gel Preparation

Prepare SDS-PAGE gels using a 12% resolving gel and a 4% stacking gel according to standard procedures. Ensure complete polymerization before use.

2. Sample Preparation

- Mix protein samples with 5× loading buffer at a ratio of 4:1 (sample:buffer);
- Typical loading volume: 20 µL sample + 5 µL loading buffer;
- Incubate at room temperature for 5 minutes;
- **Do not heat or boil membrane protein samples.**

3. Electrophoresis

- Assemble the gel in the electrophoresis tank and fill the inner chamber with 1× running buffer until wells are submerged;
- Remove comb carefully;
- Load samples: 20 µL per well;
- Load marker: 5 µL per well;
- Start electrophoresis at 120 V and run until the dye front reaches the bottom of the SDS-PAGE gel (approximately 90 minutes).

4. Staining

- Remove the gel from the plates and immerse in PAGE Staining Solution.
- Stain for 1–1.5 hours with gentle agitation.

5. Destaining

- Discard staining solution appropriately and rinse the gel briefly with water. Add destaining solution and agitate until protein bands are clearly visible.
- Stop destaining when background is sufficiently reduced
- Document the gel (e.g., imaging or photography)

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