

SODIUM DODECYL SULFATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)



Introduction

A common method for the analysis of proteins by an electrophoresis is the polyacrylamide gel based separation method. This method is also known as Sodium-Dodecyl-Sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Polyacrylamide gels prohibit the migration of large molecules in contrast to the small (faster) molecules. The internal structure of the protein must first be decomposed to be able to use this method. Adding SDS and heating the sample will cause the denaturation of the protein. Every single protein will receive a negative charge through the SDS regardless of its iso-electric point. The negatively charged proteins will move through the gel to the anode when an electric field is applied. The proteins experience resistance from the gel and get stuck in the gel when their mass is larger than the size of the pores. The proteins become visible through staining. The bands representing the proteins can be analysed for molecular mass and can be further analysed to determine which band represents which protein. Since the proteins travel only in one dimension along the gel, samples are loaded side-by-side into "wells" formed in the gel. Proteins are separated by mass into "bands" within each "lane" formed under the wells (see picture, lane 1-8). One lane is reserved for a "marker," a commercially available mixture of proteins having defined molecular weights, which is useful for size determination of proteins of interest.

Materials / reagents

- **Protein derived from cells or tissue lysate or recombinant (Pre-strained) molecular weight marker**
- **80% ethanol**
- **10% ammonium persulfate (APS)**
- **TEMED**
- **Distilled water**
- **Lysis buffer:**
 - Mix the following
 - 20 mM Tris-HCl, pH 7.4
 - 1% NP40 (non-ionic detergent)
 - 137 mM NaCl
 - 50 µM EDTA
 - Protease inhibitors (PMSF, aprotinin, Leupeptin) as needed.
- **Sample buffer (Laemmli buffer):**
 - Mix the following
 - 125 mM Tris, pH 6.8
 - 4% SDS
 - 20% Glycerol
 - 10% β-Mercaptoethanol
 - 0.0025% Bromphenol Blue
- **Tris-HCl pH 8.8 (Trisbuffer for separating gel)**
 - Measure 18.15 gram Tris
 - Add circa 50 ml water
 - Stir with the solution at least 10 minutes until homogeneous solution arises
 - Adjust pH to 8.8 with 1 M HCl
 - Fill up to 100 ml with water
 - Buffer is stable for 1 year at 4°C
- **Tris-HCl pH 6.8 (Trisbuffer for stacking gel)**
 - Measure 6.0 gram Tris
 - Add circa 50 ml water
 - Stir with the solution at least 10 minutes until homogeneous solution arises
 - Adjust pH to 6.8 with 1 M HCl
 - Fill up to 100 ml with water
 - Buffer is stable for 1 year at 4°C
- **10% Sodium dodecyl sulfate (SDS)**
 - Measure 10 gram SDS
 - Add circa 70 ml water and stir with the solution at least 10 minutes until homogeneous solution arises
 - Adjust pH to 6.8 with 1 M HCl
 - Fill up to 100 ml with water
 - Buffer is stable for 1 year at 4°C
- **10% Ammonium persulfate (APS) in water**
 - Measure 16 mg APS
 - Add 160 µl water
 - 10% APS gives best result when prepared fresh

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- Possibly it can be stored by -20°C for at most a month
- **30% Acrylamide/Bis**
 - Measure 30 g acrylamide and 0.8 g Bis
 - Add 100 ml water
 - Filter through 0.2 µm filter
 - It is advised to buy prepared 30% Acrylamide/Bis because of its neurotoxicity
- **Running buffer 10x Running buffer:**
 - Measure 144.0 gram glycine and 30.3 gram Tris
 - Add circa 600 ml water and 100 ml 10% SDS

Equipment

SDS-PAGE:

- Electrophoresis equipment (glass plates, casting stand, comb)
- Power supply
- Filter paper

Procedure

Tissue preparation

Samples may be taken from whole tissue or from cell culture. In most cases, solid tissues are first broken down mechanically using a blender (for larger sample volumes), using a homogenizer (smaller volumes), or by sonication.

Cells may also be broken open by one of the above mechanical methods. Assorted detergents, salts, and buffers may be employed to encourage lysis of cells and to solubilize proteins. Protease and phosphatase inhibitors are often added to prevent the digestion of the sample by its own enzymes.

A combination of biochemical and mechanical techniques, including various types of filtration and centrifugation, can be used to separate different cell compartments and organelles.

In case of reduced samples, boil the samples for one to five minutes in a [sample buffer](#). The boiling denatures the proteins, unfolding them completely. The SDS in the buffer then surrounds the protein with a negative charge and the β-mercaptoethanol prevents the reformation of disulfide bonds. The glycerol increases the density of the sample vs. the upper buffer in the gel tank and thus facilitates loading the samples as they will sink to the bottom of the gel pockets.

An example of preparation of cell lysates:

1. Collect cells by trypsinization and spin.
2. Lyse the pellet with 100 µl [lysis buffer](#) on ice for 10 minutes.
For 500,000 cells, lyse with 20 µl.
3. Spin at 14,000 rpm (16,000 g) in an Eppendorf microfuge for 10 min at 4°C.
4. Transfer the supernatant to a new tube and discard the pellet.
5. Determine the protein concentration.
6. Take x µl (= y µg protein) and mix with x µl of sample buffer.
7. Boil for 5 minutes.
8. Cool at ice immediately, and keep on ice.
9. Flash spin to bring down condensation prior to loading gel.

SDS-PAGE

1. Clean glass plates with ethanol and assemble casting stand, see instruction manual.
2. Mix solutions for running gel.

The percentage acrylamide used depends on the protein size:

Protein size (kD)	Percentage acrylamide/Bis acrylamide in gel
< 25	15%
25-50	12%
50-100	10%
>100	8%

These running gels can be made by mixing the following reagents:

Percentage acrylamide/Bis acrylamide in gel	15%	12%	10%	8%
Water (ml)	2.4	3.4	4.1	4.7
30 % Acrylamide/Bis (ml)	5.0	4.0	3.2	2.7

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Tris HCl pH 8.8 (ml)	2.5	2.5	2.5	2.5
10% SDS	0.1	0.1	0.1	0.1
Total volume (ml)	10	10	10	10

3. The total volume is enough for 2 gels with 0.75 mm spacer.
4. Add just before pouring the gel 50 µl 10% APS and 5 µl TEMED. In high room temperatures place the gel solution on ice to prevent early polymerization.
5. Pour the running gel solution into plates leaving about 2 cm at the top. At the top of the plates there should be sufficient room for the comb which is inserted later. There should be about 5-8 mm between the floor of the well and the top of the running gel.
6. Carefully overlay with 80% ethanol to prevent dehydration and allow to polymerize (45 min-1 hour). Once the gel has polymerized it can be wrapped in gladwrap and stored at least for several days at 4°C.
7. To continue, remove ethanol and dry in between the glass plates with filter paper.
8. Make monomer solution for the stacking gel by mixing the following reagents (enough for 2 gels).

Percentage Acrylamide/Bis in gel	4,5 %
Water (ml)	5,9
30% Acrylamide/Bis (ml)	1,5
Tris-HCl pH 6,8 (ml)	2,5
10% SDS (ml)	0,1
Total volume (ml)	10

9. Add just before pouring the gels 50 µl 10% APS and 10 µl TEMED.
10. Overlay running gel and insert comb carefully to prevent air bubbles.
11. Allow to set for 30-45 minutes. Generally the stacking gel should not be prepared until the samples are ready as there is a pH difference between the two gels which will diffuse with time.
12. Assemble running unit, see instruction manual provided by the manufacturer. Make sure that there are no air bubbles and contaminations (salt excess) in the well or around the gel. This should be done just before samples are ready as the wells will dry out quite quickly.

Use of positive controls and molecular weight marker

To monitor the progress of an electrophoretic run a range of molecular weight (MW) markers with determined protein size should be used. A range of MW markers are commercially available. Prestained markers are recommended for convenience.

13. Apply 5-20 µg total proteins of cell or tissue lysate to each well of a 0.75–1.0 mm thick gel. For thicker gels (1.5 mm thick), apply up to 25-40 µg in each well.
14. Use special gel loading tips or a micro-syringe to load the complete sample in a narrow well with limited movement at the bottom of the well, resulting in high quality data. Never overfill wells. This could lead to artefacts.
15. Run the gel with constant current for the recommended time as instructed by the manufacturer (1 hour to overnight).
16. Electrophorese until the bromophenol blue reaches the bottom of the gel. Turn off power supply. Keep gels in running buffer until ready to transfer.

Continue immediately with either staining of the gel or with Western Blotting to prevent diffusion of proteins in the gel.

Safety

- Under no circumstances shall Hbt be liable for any damage arising from the use of this protocol. User should be trained and be familiar with the test procedure.
- Samples of tissue, serum or blood origin should be handled to guidelines for prevention of transmission of blood borne diseases.
- Some enzyme substrates are considered hazardous, due to potential carcinogenicity. Handle with care and refer to Material Safety Data Sheets for proper handling precautions.
- Wear appropriate protective clothing, gloves, and eyewear to avoid any accidental contact with reagents.
- Reagents which contain preservatives may be toxic if ingested, inhaled, or in contact with skin.
- The waste-disposal should be performed according to your laboratories regulations.
- SDS is a neurotoxin and harmful when ingested and irritating for eyes and skin. Do not inhale the material. Avoid contact with eyes and skin.
- β-Mercaptoethanol is a harmful and poisonous material. Avoid contact with eyes and skin. It has a very penetrating smell.
- Acrylamide is a potent cumulative neurotoxin: wear gloves at all times!

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- Pay attention that you do not inhale acryl amide powder.

Notes

- Please note that this is a general protocol.
- This protocol is to be used for research purposes only.
- The appropriate negative and positive controls should be included in every trial.
- Standards must be run with each assay to ensure accuracy.

Technical assistance

For technical assistance and more information please contact Hycult Biotech directly at support@hycultbiotech.com

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