Experiment 04: Protein Electrophoresis (SDS-PAGE)

Principle:

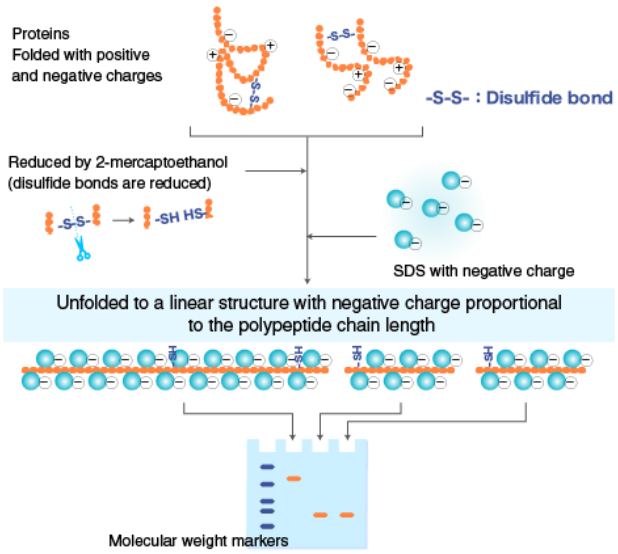
Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is the most desirable method used for the qualitative analysis of the protein mixtures. This method is basically used for checking purity of the proteins. Since, in SDS-PAGE the proteins get separated in accordance to their size, the method is also used to determine the molecular weight of the proteins. After performing the SDS-PAGE, subsequent specialized techniques such as Western blotting, two-dimensional gel electrophoresis and peptide mapping can be done.

When proteins are separated by electrophoresis through a gel matrix, smaller proteins migrate faster due to less resistance from the gel matrix. Other influences on the rate of migration through the gel matrix include the structure and charge of the proteins.

In SDS-PAGE, the use of sodium dodecyl sulfate (SDS, also known as sodium lauryl sulfate) and polyacrylamide gel largely eliminates the influence of the structure and charge, and proteins are separated solely based on polypeptide chain length.

SDS is a detergent with a strong protein-denaturing effect and binds to the protein backbone at a constant molar ratio. In the presence of SDS and a reducing agent that cleaves disulfide bonds critical for proper folding, proteins unfold into linear chains with negative charge proportional to the polypeptide chain length.

Polymerized acrylamide (polyacrylamide) forms a mesh-like matrix suitable for the separation of proteins of typical size (10-180kD). The strength of the gel allows easy handling. Polyacrylamide gel electrophoresis of SDS-treated proteins allows researchers to separate proteins based on their length in an easy, inexpensive, and relatively accurate manner.



Materials:

Acrylamide/Bisacrylamide-stock solution (30: 0~8 **%**),

Separation gel butter, stacking gel butter,

10 x running buffer-concentrate

Tetramethylendiamine (TEMED) solution,

Incubation buffer,

Ammoniumpersulphate (APS, calibrated tube (10 ml)

Staining Solution: 100 ml staining solution

Coomassie Brilliant Blue R250 0.25 g

Glacial acetic acid 10 ml

MeOH: H2O (1: 1 v/v) 90 ml)

Destaining Solution: 100 ml destaining solution

Glacial acetic acid 10 ml

MeOH: H2O (1: 1 v/v) 90 ml

Procedure (all given amounts are in ml)

Please note

The brown light protected bottle with Acrylamid/Bisacrylamid Stock solution contains a special stabilizing granulate to prohibit formation of neurotoxic acrylic acid during storage. To avoid mixing it into the gel solution let it settle for approx. 15 seconds before decanting PAA stock-solution!

1. Mix samples (stored in aliquots at 20°C) and marker proteins 1: 1 with incubation buffer and incubate 2 min. at 100°C! Always centrifuge the samples prior to gel application to remove undissolved particles

2. Ammoniumpersulphate solution (10%, **w/v)** should be prepared freshly every week using the enclosed

caIibrated 1 tube (1 g APS 3d 10 ml Aqua bid.) Store APS-Solution at 4°C.

Attention: Acrylamide is a neurotoxine - always wear gloves when handling or casting polyacrylamide gels!

3.

Pipet scheme for 15 ml separation gel	5%	7,5%	10%	12,5%	15%
PAA Stock solution	2.50	3.75	5.00	6.25	7.50
Separation gel buffer	5.00	5.00	5.00	5.00	5.00
Aqua bidest.	7.25	6.00	4.75	3.50	2.25
TEMED~ 10 %	0.25	0.25	0.25	0.25	0.25

Mix the solution well before adding ammoniumpersulphate (APS)! Add 0.1 ml Ammoniumpersulphate Solution (10%). Mix well and use at once

4. Pipet scheme tor 5 ml stacking gel:

	3%	8%
PAA stock solution	0.50	1.00
stacking gel butter	1.00	1.00
Aqua bidest.	3.40	2.90
TEMED 10 %	0.10	0.10

Mix the solution well before adding ammoniumpersulphate (APS)!

Add 0.03 ml Ammoniumpersulphate Solution (10%). **Mix well and use at once!**

Let the gels polymerize at least 1 hour prior to use! Cast gels can be stored in a wetted plastic bag for up to two weeks at 4-8°C until use.

5. Dilute running buffer-concentrate 1 + 9 parts with Aqua bidest, fill into electrophoretic chamber and install glass plate/gel sandwich without catching air bubbles.

6. Apply electric current to the electrophoretic chamber by an authorized power supply (CE-, lso-certified). Set voltage to 200 - 250 volts (V) and current to 20 - 25 mA for one gel 8Ox8Oxl mm, 10% PAA. Stop the run after blue front dye (Bromephenole-blue) has reached bottom of the gel (after approx. 1 - 1.5 hours).

7. Remove the gel from the electrophoretic chamber, disassemble the glass plate/ gel sandwich diligently.

8. Incubate the gel in staining solution with shaking for 30 min or longer (can leave it overnight).

9. Remove the dye solution (it can be reused for many times) and rinse the gel with water 1-2 times to remove the dye.

10. Add destaining solution to the gel and incubate for 30-60 min.

11. Transfer the gel to water, visualize in gel doc.

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