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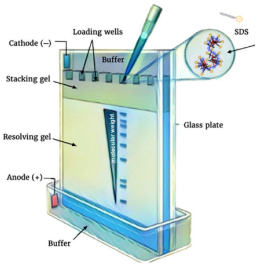
SDS page



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Protocol status: Other
The protocol is developed based on literature and has not been tested yet.

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Abstract

SDS-PAGE is an analytical technique used to separate proteins based on their molecular weight using electrophoresis. Peptides migrate faster due to less resistance from the gel matrix. The peptide used in this experiment is characterised by a short length (molecular weight is less than 3kDa), so we have used a very dense (20%) gel in order to get a clear separation.

Image Attribution

https://www.researchgate.net/figure/Schematic-of-SDS-Page-electrophoresis-Polyacrylamide-two-part-gel-composed-of-a-stacking_fig21_315866219

Guidelines

Composition of Stacking Gel (4 %) 2 gels
DDI H2O 3.9 ml
1.0 M Tris-HCl, pH 6.8 (SG Bfr.) 500 µl
40% Acrylamide Stock 500 µl
20 % SDS 100 µl
30% Ammonium Persulfate 16 µl
TEMED 8 µl

Composition of Mini Protean II SDS Gel (15%) 2 gels
DDI H2O 3.7ml
1.5 M Tris-HCl, pH 8.8 (RG Bft.) 4ml
40% Acrylamide Stock 8.0 ml
10 % SDS 160µl
10% Ammonium Persulfate 160µl
TEMED 16µl

Note

Ammonium Persulfate and TEMED is to be added immediately before casting gel. Number of gels refers to 1 mm thick gels. The 2 gel recipe is to be used for 1.5 mm thick gels.

Troubleshooting

SDS PAGE of a very small protein

1 **Casting the gel:**

1] Glass plates and spacers will be assembled in gel casting apparatus–see BioRad instruction manual.

2] The components will be mixed for the resolving gel as described in the subscript.

3] The resolving gel mixture will be poured into the gel plates to a level 2 cm below the top of the shorter plate.

4] A layer of DDI H2O will be paced over the top of the resolving gel to prevent meniscus formation in the resolving gel.

5] Resolving gel will be allowed to stand 00:30:00 at room temperature.

6] The DDI H2O will be drained from top of the resolving gel, rinsed with DDI H2O, drain, and any remaining DDI H2O is to be wicked away with a Kimwipe.

7] Components will be mixed for stacking gel.

8] Stacking gel solution will be poured into gel plates (on top of running gel), so that gel plates are filled. Comb is to be inserted to the top of the spacers.

9] The gel is allowed to stand for at least 01:00:00 at room temperature, or overnigt at 4 °C (wrapped in saran wrap).

2 **Preparing Samples:**

Note

Note: 10 well combs will hold up to 30 µL of prepared sample. 15 well combs will hold up to 20 µL of prepared sample.

2.1 **Solution Samples**

1] A volume of protein solution (or 1 µL of standard) is to be placed into a µfuge tube, such that there is 10 mg of protein in the solution.

2] An equal volume of 2x sample buffer (or 10 µL for standards) will be added.

3] Tubes will be incubated in boiling water for 00:05:00 .

4] Will be centrifuged at 12,000 x g for 00:00:30 .

3 **Running the Gel**

1] Comb will be removed and cast gel will be assembled into Mini-Protean II apparatus.
2] Freshly prepared 1x running buffer (300 mL) will be added to both chambers of the apparatus.
3] The prepared samples will be loaded into the wells of the gel.
4] The gel will be run at 100 V until the dye front migrates into the running gel (~ 00:15:00), and increased to 200 V until the dye front reaches the bottom of the gel (~ 00:45:00).

4 **Staining & Destaining the Gel**

1] The run gel is to be removed from the aparatus and the spacers and glass plates to be removed too. The gel will be placed into a small tray.

Note

Note: Never use a metal spatula to separate the glass plates.

2] ~ 20 mL staining solution will be added and stained for > 00:30:00 with gentle shaking.
3] Will be poured off and stain saved.
4] 5 mL destain solution to be added and destained for approximately 00:01:00 with gentle shaking.
5] Will be poured off and the destain solution to be discarded. ~ 30 mL of destain solution to be added.
6] Will be destained with gentle shaking until the gel is visibly destained (> 02:00:00).
7] Will be poured off and the destain solution will be discarded.
8] DDI H2O is to be used for rinsing. ~ 30 mL DDI H2O to be added and rinsed for 00:05:00 with gentle shaking.
9] The gel will be dried on the gel dryer at 60 °C for 01:00:00 with a sheet of Whatman filter paper below the gel and a piece of Seran wrap over the gel.