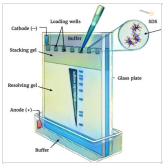

Oct 24, 2020

SDS page

Andreea S[1]

[1]University of Groningen

Other dx.doi.org/10.17504/protocols.io.bkktkuwn

iGEM Groningen 2020

s.sarkar.2

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.

DOI

dx.doi.org/10.17504/protocols.io.bkktkuwn

PROTOCOL CITATION

Andreea S 2020. SDS page . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bkktkuwn

IMAGE ATTRIBUTION

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

CREATED

Sep 01, 2020

LAST MODIFIED

Oct 24, 2020

PROTOCOL INTEGER ID

41331

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

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.

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.

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: 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** .

Citation: Andreea S (10/24/2020). SDS pageÃÂ . https://dx.doi.org/10.17504/protocols.io.bkktkuwn

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: 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.

Citation: Andreea S (10/24/2020). SDS pageÃÂ . https://dx.doi.org/10.17504/protocols.io.bkktkuwn