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SDS-PAGE gel electrophoresis V.2

Steven J Burgess[1]

[1]University of Illinois at Urbana-Champaign

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Ag SynBio Lab UIUC

Steven Burgess
University of Illinois at Urbana-Champaign

ABSTRACT

SDS-PAGE gel electrophoresis protocol for analyzing samples form field-grown tissue via immunofluorescence. In this protocol no Coomassie blue is added to samples, the reason is that this interferes with the fluorescent signal during immunoblot. Instead, samples have already been prepared in Laemmli buffer (minus coomassie, see protein extraction procedure), the leading edge of samples can be visualized due to the presence of chlorophyll.

Note

- When using 15 well, 0.75 mm comb, try to limit the volume loaded to 10 µL to minimize the risk of spillover of protein between wells.
- Ensure to wipe the tip on the rim of the sample tube to remove the sample stuck to outside of the tip.

Literature:

http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin_6040.pdfhttps://www.bio-rad.com/webroot/web/pdf/lsr/literature/10026447.pdf

PROTOCOL CITATION

Steven J Burgess 2020. SDS-PAGE gel electrophoresis. **protocols.io**
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MATERIALS TEXT

- 4–20% Mini-PROTEAN® TGX™ Precast Protein Gels, 15-well, 15 µl (Bio-Rad Laboratories; 4561096)
- Opening lever (Bio-Rad Laboratories; 456-0000)
- Chameleon™ Duo Pre-stained Protein Ladder (LI-COR Biosciences; NC0738562)
- Mini-PROTEAN® Tetra Vertical Electrophoresis Cell, 4-gel (Bio-Rad Laboratories; 1658004)
- 10x Tris/Glycine/SDS (Bio-Rad Laboratories; 1610732)
- Fisherbrand™ Gel-Loading Tips, 1-200 µL (Fisher Scientific; 02-707-181)
- Imperial™ Protein Stain (Thermo Scientific; 24615)s
- Bio-Rad Gel-Doc Imager (optional)

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Prepare gel tank and buffers

1 Create a working dilution of Tris-Glycine running buffer (~ **1 L** is required per gel tank) by diluting 1:10 with d H_2O.

2 Carefully remove the comb from the precast gel and the tape across the bottom.

3 Assemble the Mini-PROTEAN electrophoresis cell and fill the inner chamber with buffer and the outer chamber up to the recommended mark

the volume varies depending on whether running 2 or 4 gels, the level is marked on the tank

4 Wash the wells with running buffer by pipetting up and down

This is done to remove residual acrylamide that may have collected in wells

Prepare Samples 10m

5 In fresh centrifuge tubes, create a dilution of each sample using 1x PEB, such that each sample is set at a concentration of 3 µg /mL of total soluble protein.

Recommended final volume ~ **100 µl** µL (this will allow for 10 samples) but will depend on the application

6 Heat samples at **95 °C** for **00:05:00** . 5m

7 Spin down samples at **10000 x g** for **00:05:00** to pellet debris. 5m

8 Load **3 µl** of Chameleon™ Duo Pre-stained Protein Ladder to the first well

9 Load 10 µL of each sample (30 µg of total soluble protein) per lane.

Running Gel 10m

10 Run precast gels at 200 V for ~ 00:30:00 . 30m

Or until the samples have reached the end of the gel. For self-made gels, run at 80-120 V

11 Carefully open precast gel case using an opening lever, by inserting where the black arrows indicate on the gel case.

12 Remove stacking gel with a blade

13 Proceed either directly to immunoblot, or if the gel is as a loading control, place in a container and cover with Imperial™ Protein Stain.

Visualizing Gel (optional) 10m

14 Gently agitate on a rocking platform for 00:30:00 . 30m

15 Pour off the InstantBlue stain (collect as hazardous waste)

16 Rinse the gel with dH_2O.

17 Cover the stained gel with dH_2O and gently agitate in a rocking platform for 00:30:00 . 30m

18 Repeat steps 15-17 until the background of the gel is clear and blue protein bands can be clearly visualized

19 Image the gel on the Bio-Rad Gel Doc system trans-white illumination