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SDS-PAGE gel electrophoresis V.4

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SDS-PAGE gel electrophoresis protocol for analyzing samples from plant leaf 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, 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 that accurate volume is pipetted by removing sample stuck to the outside of the pipette tip by wiping the tip on the rim of the sample tube to remove any residual liquid.

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

DOI

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Lynn Doran

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- 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)

Extract protein from samples via Leaf Protein Extraction for Immunoblot and determine Protein Concentration using Qubit 4 Fluorometer.

Previously extracted and quantified protein samples can be stored at **-20 °C** .

Prepare gel tank and buffers

1 - Create a 1X working dilution of Tris/Glycine/SDS buffer (~ **1 L** is required per gel tank) by diluting 10X stock 1:10 with distilled H_2O.

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

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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 microcentrifuge tubes, create a dilution of each sample using 1x PEB to a concentration of 3 µg /uL of total soluble protein.

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

Heating previously frozen protein extracted samples at 5 **50 °C** for **00:05:00** can help resolubilization of SDS in protein extraction buffer prior to making dilutions.

6 Briefly vortex the sample to shear any DNA contamination.

7 Load **3 µL** of Chameleon™ Duo Pre-stained Protein Ladder to the first well.

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

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Running Gel 10m

9 Run precast gels at 200 V for ~ 00:30:00 or until the samples have reached the end of the gel. In some applications it may be advantageous to run the chlorophyll off the end of the gel to improve fluorescence and signal on the protein of interest. 30m

Mini-PROTEAN Tetra Cell
Gel Electrophoresis Tank
Bio-rad Laboratories 1658005EDU

For self-made gels, run at 80-120 V.

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

11 Remove stacking gel with a blade

12 Proceed either directly to Protein Transfer using Bio-rad TransBlot Turbo or Total Protein Staining.

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