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We use this protocol and it's working

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SDS-PAGE gel electrophoresis

Forked from SDS-PAGE gel electrophoresis

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ABSTRACT

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

MATERIALS

- 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)

BEFORE START INSTRUCTIONS

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.

> **Note**
>
> Pull the comb straight back to avoid damaging the thin well structure of the gel.

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

> **Note**
>
> 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

Note

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 pre-determined concentration total soluble protein.

Note

Recommended final concentration is 3 ug/ul of soluble protein for a 10 ul/well total of 30 ug protein, however this value will need to be determined empirically using a linear response curve for each antibody and will vary depending on the loading volume as determined by the well sizes. final volume ~ 100 µL (this will allow for 10 samples) but will depend on the application.

Note

Heating previously frozen protein extracted samples at 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.

Note

See Note above about sample concentrations and well volumes.

Running Gel

10m

9 Run precast gels at 170V for ~ 00:50:00 or until the samples have reached the end of the gel. 50m
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.

Equipment

Mini-PROTEAN Tetra Cell NAME

Gel Electrophoresis Tank TYPE

Bio-rad Laboratories BRAND

1658005EDU SKU

https://www.bio-rad.com/en-us/product/mini-protean-tetra-cell?ID=d5d6580e-b8f6-4f1c-bbc4-f63d360ea788 LINK

Note

For self-made gels, run at 80-120 V. Voltage may need to be varied based on number of gels in the tank. If bending of lanes is observed, with increased bending in the center, try reducing the voltage and increasing the run time. Bending is often a by-product of the gel melting due to too high of electricity.

10 Carefully open precast gel case using an opening lever, by inserting where the black arrows indicate on the gel case. Use the green Bio-rad scraper to cut the wells off of the gel. Also use the scraper to remove the bottom edge of the gel that was below the union of the two pieces of plastic.

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