VERSION 3

DEC 09, 2020

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bqiemube

Protocol Citation: Steven J Burgess 2020. SDS-PAGE gel electrophoresis. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bqiemube> Version created by Steven J Burgess

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Protocol status: In development
 We are still developing and optimizing this protocol

Created: Dec 08, 2020

Last Modified: Dec 09, 2020

PROTOCOL integer ID:
 45350

SDS-PAGE gel electrophoresis V.3

In 1 collection

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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 to wipe the tip on the rim of the sample tube to remove the sample stuck to the outside of the tip.

Literature:

http://www.biorad.com/webroot/web/pdf/lsr/literature/Bulletin_6040.pdf
<https://www.biorad.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)

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 H₂O.
- 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

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 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.

Note

Recommended final volume ~ 100 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

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

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 protein transfer

Protocol

NAME

Protein Transfer

CREATED BY

Steven J Burgess

PREVIEW

or if the gel is as a loading control, place in a container and cover with

- 13.1** Place the membrane and bottom stack in the middle of the cassette base
- 13.2** Place pre-cast gel in the middle of the membrane and roll to remove air-bubbles
- 13.3** Place the top stack on top of the gel, gently roll
- 13.4** Close the cassette lid, taking care not to disturb the gel
- 13.5** Place the cassette in the Trans-Blot Turbo and follow the instructions on the machine (Fast protocol TGX gel).

Equipment

Trans-Blot® Turbo™

NAME

Protein transfer apparatus

TYPE

Bio-rad Laboratories

BRAND

1704150EDU

SKU

<https://www.bio-rad.com/en-us/product/trans-blot-turbo-transfer-system?ID=M7FAX015>

LINK

- 13.6** Either dry membrane and store at 4 °C for later use or proceed immediately to fluorescent western protocol

Visualizing Gel (optional)

10m

- 14** Gently agitate the gel on a rocking platform for 00:30:00 . 30m
- 15** Pour off the Imperial Protein Stain (collect as hazardous waste)
Imperial™ Protein Stain **Thermo Fisher Catalog #24615**
- 16** Rinse the gel with dH₂O.
- 17** Cover the stained gel with dH₂O 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 XR system trans-white illumination

Equipment	
Gel Doc XR+ Gel Documentation System	NAME
Gel Documentation System	TYPE
Bio-rad Laboratories	BRAND
1708195	SKU
https://www.bio-rad.com/en-us/product/gel-doc-xr-gel-documentation-system?ID=O494WJE8Z	LINK