



Version 3 ▼ Dec 09, 2020

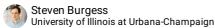
# SDS-PAGE gel electrophoresis V.3

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In Development This protocol is published without a DOI.

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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  $\mu$ L 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 theoutside of the tip.

### Literature:

http://www.bio-rad.com/webroot/web/pdf/lsr/literature/Bulletin\_6040.pdfhttps://www.biorad.com/webroot/web/pdf/lsr/literature/10026447.pdf

PROTOCOL CITATION

Steven J Burgess 2020. SDS-PAGE gel electrophoresis. protocols.io https://protocols.io/view/sds-page-gel-electrophoresis-bqiemube Version created by Steven Burgess

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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<sup>TM</sup> Protein Stain (Thermo Scientific; <u>24615</u>)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 <sub>2</sub>	<u>:</u> O.		
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 recommended mark	) the		
	the volume varies depending on whether running 2 or 4 gels, the level is marked on the tank			
	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 concentrate of 3 $\mu$ g /mL of total soluble protein.	atior		
	Recommended final volume ~ <b>□100 μI</b> (this will allow for 10 samples) but will depend on the application			
6	Heat samples at 8 95 °C for © 00:05:00	5m		
7	Spin down samples at <b>⊕10000 x g</b> for <b>⊕00:05:00</b> to pellet debris	5m		

Load **□3 µI** of Chameleon<sup>™</sup> Duo Pre-stained Protein Ladder to the first well

Load 10 μl of each sample (30 μg of total soluble protein) per lane Running Gel 10m 30m 10 Run precast gels at 200 V for ~ © 00:30:00 . Mini-PROTEAN Tetra Cell Gel Electrophoresis Tank Bio-rad Laboratories 1658005EDU Or until the samples have reached the end of the gel. For self-made gels, run at 80-120 V 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 Proceed either directly to protein transfer 13 Protein Transfer PREVIEW RUN by Steven Burgess, University of Illinois at Urbana-Champaign or if the gel is as a loading control, place in a container and cover with ⋈ Imperial™ Protein Stain Thermo Fisher Catalog #24615 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

Place the top stack on top of the gel, gently roll

13.3

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).	
	Trans-Blot® Turbo™ Protein transfer apparatus Bio-rad Laboratories 1704150EDU ←	
13.6	Either dry membrane and store at 8 4 °C for later use or proceed immediately to fluorescent western protocol	
Visualiz	ring 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)	
16	Rinse the gel with $dH_2O$ .	
17	Cover the stained gel with dH $_2$ O and gently agitate in a rocking platform for $©$ <b>00:30:00</b>	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	
	Gel Doc XR+ Gel Documentation System Gel Documentation System Bio-rad Laboratories 1708195 🖘	