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SDS PAGE & Western Blot for Membrane proteins [↗](#)

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1 Works for me [dx.doi.org/10.17504/protocols.io.mhtc36n](https://doi.org/10.17504/protocols.io.mhtc36n)

CyanoWorld



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EXTERNAL LINK

<http://www.bio-rad.com/webroot/web/pdf/lsr/literature/10026389.pdf>

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

null

□
Clarity Western ECL
substrate.pdf

□
Mini-PROTEAN®Precast
gels.pdf

□
Trans-Blot Turbo Blotting
System.pdf

MATERIALS

NAME ▼	CATALOG # ▼	VENDOR ▼
2 M Tris-Cl, pH 6.8		
DTT (1,4-Dithiothreitol)	10708984001	Sigma Aldrich
Glycerol - for molecular biology, ≥99%	G5516	Sigma Aldrich
20% SDS		
Bromophenol blue		
PageRuler Prestained Protein Ladder, 10 to 180 kDa	View	Thermo Fisher Scientific
Soy Milk (Alpro Soya original)	View	
10 x TGS buffer	1610732	BioRad Sciences

Prepare 4 x loading dye

- | 1 | For 10 mL: | final conc. |
|---|------------------------------|-------------|
| | 617 mg DTT | 400 mM |
| | 1,25 mL 2M Tris-Cl (pH 6.8) | 250 mM |
| | 4 mL Glycerol | 40% (w/v) |
| | 4 mL 20% SDS | 8% (w/v) |
| | ad H ₂ O to 10 mL | |



Work under the fume hood and wear gloves!

DTT (Dithiothreitol) is flammable and harmful if swallowed or inhaled. Maybe harmful if absorbed through the skin. Causes irritation to skin, eyes and respiratory tract.

SDS (sodium dodecyl sulfate) causes eye and skin irritation and may cause respiratory tract irritation.

Prepare TBS-T buffer

2 Stock solutions:

- 1) 1 M Tris (pH 7.5) - for 1 L: **121,14 g Tris**, titrate with **HCl**
- 2) 5 M NaCl - for 500 mL: **146,1 g NaCl**

For 1 L TBS-T:

100 mL 1 M Tris (pH 7.5)
30 mL 5 M NaCl
1 mL Tween 20



When using concentrated **HCl (37%)**, work in the hood! HCl 'smoke' attacks your lungs when inhaled!

Sample denaturation

- 3
 - adjust protein samples to equal concentrations by adding PBS
 - add 1 Vol. loading dye (LD) to 3 Vol. sample:

e.g.:

2.5 µL LD + 7.5 µL sample
4 µL LD + 12 µL sample
5 µL LD + 15 µL sample

- incubate 30 min at 50 °C



Do *not* *boil*, in order to prevent aggregation !

⚠ 50 °C

🕒 00:30:00

Gel loading

- 4
 - spin down denatured samples for 1 min at 12000 g
 - assemble 'Mini-Protean' chambers and add a *mock plate* if required
 - fill cathode reservoir and chamber (until marker) with 1 x TGS buffer (Biorad)
 - remove comb and bottom tape from 'Mini Protean TGX precast gel'
 - carefully load sample supernatants into wells using Hamilton syringe or pipette
 - load 4 µL 'PageRuler prestained Protein ladder' into one well



Work under the fume hood and wear vinyl/PVC **gloves** ! (cf. step 1)

Acrylamide (non-polymerized) is a neurotoxin which can be absorbed orally, dermally or by inhalation. Signs of exposure include tingling in the extremities and/or loss of coordination.

Electrophoresis

- 5
 - run gel at constant current of 20-40 mA

Western Transfer

- 6
 - assemble blotting sandwich according to the Trans-Blot Turbo Transfer System- Quick Sart Guide (link below)
 - **take care of removing all bubbles (!)**
 - close and lock cassette lid
 - insert cassette into the instrument

Transfer conditions:

Program '**Standard SD**' - 30 min (up to 1.0 A; 25 V constant)

<http://www.bio-rad.com/webroot/web/pdf/lsr/literature/10016505D.pdf>

Staining

7

Blocking

- 8
 - Incubate 15 min with **soy milk** at room temperature

cf.: *Analytical Biochemistry* 426 (2012) 22–23 (link below)

<https://doi.org/10.1016/j.ab.2012.03.028>

Washing/ Equilibration

- 9
 - incubate in TBS-T for 3 x 15 min at room temperature

Primary antibody

- 10 **for antibodies against DevBCA, TolC:**

- prepare 1:5000 dilution in TBS-T
- incubate at 4 °C overnight under gentle shaking

Washing

- 11 ■ incubate in TBS-T for 3 x 15 min at room temperature

Secondary antibody

- 12 ■ prepare 1:5000 dilution of anti-rabbit antibody
 ■ incubate for 1 hour at room temperature

Washing

- 13 ■ incubate in TBS-T for 3 x 15 min at room temperature
- 14 ■ Detection kit: chemiluminescent detection of HRP-conjugates was performed with the “Clarity Western ECL” kit (Biorad)
 ■ Detection system: ChemiDoc XRS system (Biorad) with cumulative image recording using the Quantity One software (Biorad).



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