Instructional Protocol for PrecisionAb™ Antibodies Customers

Protocol

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MATERIALS

- Protein samples and reagents
  - Endogenous lysates
  - Reducing agents such as dithiothreitol (DTT) or β-mercaptoethanol (BME)
- Electrophoresis gels, reagents, and equipment
  - 4–15% Mini-PROTEAN® TGX Stain-Free™ Precast Gels (10 well, 50 µl), Bio-Rad cat. #456-8084
  - Any kD™ Mini-PROTEAN® TGX Stain-Free™ Precast Gels (10 well, 50 µl), Bio-Rad cat. #456-8124
  - Mini-PROTEAN Tetra Cell for Mini Precast Gels, Bio-Rad cat. #165-8004
  - PowerPac™ Universal Power Supply, Bio-Rad cat. #164-5070
  - Precision Plus Protein™ All Blue Standards Value Pack, Bio-Rad cat. #161-0393
  - 1x Tris/glycine/SDS (TGS; running buffer), Bio-Rad cat. #161-0772
  - 2x Laemmli sample buffer, Bio-Rad cat. #161-0737
- Transfer membranes, reagents, and equipment
  - Trans-Blot® Turbo™ Transfer System, Bio-Rad cat. #170-4150
  - Trans-Blot Turbo Mini PVDF Transfer Pack, Bio-Rad cat. #170-4156
- Western blotting reagents and equipment
  - Primary antibodies (from the AbD Serotec® catalog)
  - Secondary antibodies (see antibody datasheet)
  - 10x Tris-buffered saline (TBS), Bio-Rad cat. #170-6435
  - 10% Tween 20, Bio-Rad cat. #161-0781
  - 1x TBS + 1% casein (blocking buffer), Bio-Rad cat. #161-0782
  - 1x TBS + 0.1% Tween 20 (TBST)
- Imaging reagents and equipment
  - ChemiDoc™ MP System, Bio-Rad cat. #170-8280
  - Clarity™ Western ECL Substrate, Bio-Rad cat. #170-5061
  - Image Lab™ Software (v5.2 or greater), Bio-Rad cat. #170-9690

PrecisionAb Antibodies have been extensively validated for western blotting. This protocol describes how to use PrecisionAb Antibodies to get the best western blotting results. At the end of the protocol, there are details for more resources that will provide comprehensive procedures and guidance to produce successful western blots.

1 Lysate preparation

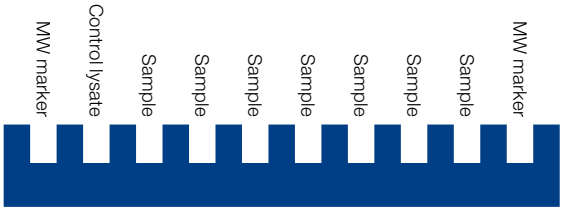
Reconstitute the 400 µg lysate in one of the following ways, depending on the reducing reagent used:

- If using DTT, add 190 µl H_20, 200 µl 2x Laemmli Sample Buffer, and 10 µl 2 M DTT
- If using BME, add 180 µl H_20, 200 µl 2x Laemmli Sample Buffer, and 20 µl BME

Heat at 95°C for 5 min.

2 Gel Electrophoresis

Load the control cell lysate adjacent to your samples and the molecular weight (MW) marker (see diagram).



The amount of control lysate loaded is dependent on the gel comb size. Please see the recommendations in the tables below.

Mini gels (8.6 cm x 6.7 cm)

Description	10-Well 30 µl	10-Well 50 µl	15-Well 15 µl	12-Well 30 µl	8+1-Well 30 µl
Recommended loading volume, µl	25	25	15	20	25

Midi gels (13.3 cm x 8.7 cm)

Description	12+2-Well 45 µl	18-Well 30 µl	26-Well 15 µl
Recommended loading volume, µl	35	25	15

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3 Blocking

During the validation process, we blocked for 30 min at room temperature (RT) in blocking buffer + 0.1% Tween 20. When using casein, do not block for longer than 30 min to prevent reduction in signal specificity. We recommend using casein or nonfat dried milk for blocking. If using BSA, you may notice some nonspecific bands due to its low stringency. Please see the validation protocol (bulletin 6603) for more details.

4 Incubation with Primary Antibody

- Dilute the primary antibody 1:1,000 in 10 ml blocking buffer
- Incubate the blot in the primary antibody and blocking buffer solution at 4°C overnight with gentle agitation
- Rinse the blot with 15 ml TBST at RT for 5 min. Repeat for a total of five washes

5 Incubation with Secondary Antibody

- Rinse the blot with 15 ml TBST at RT for 5 min. Repeat for a total of five washes
- Dilute the appropriate secondary antibody in 10 ml blocking buffer according to the following table:

Recommended secondary antibody dilutions.

Primary Antibody Host	Secondary	AbD Serotec Catalog #	Recommended Dilution
Mouse	GAM-HRP	STAR207P	1:10,000
Rabbit	GAR-HRP	STAR208P	1:10,000
HuCAL®	Goat anti-human IgG F(ab')2:HRP	STAR126P	1:2,500
Rat	Goat anti-rat IgG:HRP	STAR72	1:10,000
Goat	Donkey anti-sheep/goat IgG:HRP	STAR88P	1:10,000
Sheep	Rabbit anti-sheep IgG:HRP	5184-2504	1:10,000

Please refer to the antibody product page at **abdserotec.com** for details on the exact secondary antibody used during the validation process.

- Incubate the blot in the secondary antibody and blocking buffer solution at RT for 1 hr with gentle agitation
- Rinse the blot with 15 ml TBST at RT for 5 min. Repeat for a total of five washes. After the final wash step, keep the blot in TBST while preparing for blot detection

6 Blot Detection

All PrecisionAb Antibodies were validated using enhanced chemiluminescent (ECL) detection. It is important to use an ECL substrate that has good sensitivity and long signal duration, such as the Clarity Western ECL Substrate.

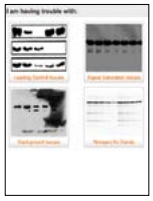
- Mix the Clarity Western ECL Substrate Kit components in a 1:1 ratio. Prepare 0.1 ml solution per cm^2 of membrane
- Place the membrane, protein side down, in the substrate solution, and let the membrane develop for 5 min

Additional Resources

For more western blotting tips, please use the following resources:

Bulletin 2895

Western blotting overview



Troubleshoot western blots

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10048952 Rev A US/EG *14-2118 1214 Sig 1213*