Short Protocol

PROMETHEUS™
Protein Biology Products

For Catalog Numbers

Cat # 20-302 100 ml, sufficient for 1000 cm^2

Cat # 20-302B 200 ml, sufficient for 2000 cm^2

ProSignal™ Femto

Storage Information

The ProSignal Femto reagents are stable at room temperature for at least one year. Reagents do NOT require refrigeration. Storing reagents refrigerated may DECREASE shelf life. Refer to detailed manual for more information.

Warnings and Precautions:

- ProSignal Femto is for research use only.
- Always wear gloves when handling membranes and reagents.
- Refer to MSDS for additional safety information.
- The product is guaranteed to be free of manufacturer defects, and to function as described when stored appropriately, and the provided protocol is followed by properly trained personnel.

Short Protocol

Step
1. Prepare your protein blot
2. Block the membrane for one hour at room temperature (RT)
3. Incubate the blot with a primary antibody for one hour at RT with gentle agitation
4. Wash blot: • 1 x quickly • 1 x 15 min, with 0.7 ml/cm^2 membrane • 3 x 5 min, with at least 0.3 ml/cm^2 membrane each time
5. Incubate the blot with a secondary antibody for one hour at RT with gentle agitation
6. Wash blot: • 3 x 5 min, with at least 0.3 ml/cm^2 membrane each time
7. Mix ProSignal Femto components 1:1 in sufficient amounts to obtain 0.1 ml/cm^2 of your membrane and place on the blot for 2 minutes.
8. Drain excess reagent
9. Image the blot by CCD camera or cover with plastic wrap and expose to X-ray film

Troubleshooting & FAQ

Western blotting can require substantial optimization due to the multiple steps involved. The correct amount of protein to load on the gel and the best dilutions of primary and secondary antibodies must be determined empirically. Some common questions are addressed below:

Problem	Possible Solutions
High background	• Reduce primary antibody concentration by increasing the dilution factor. • Try a different blocking buffer. • Try a shorter exposure time. • Increase washing time and/or number of washing steps.
No or low signal	• Check that correct primary antibody used. • Check that secondary antibody recognizes primary (for example, if the primary is a rabbit antibody, that the secondary is goat-anti-rabbit).
White spots within bands	• Improve transfer, making sure to remove any bubbles between the gel and the membrane.
Speckled background	• Filter blocking and washing buffers. • Ensure that the laboratory environment is clean, to minimize dust, debris or any other particles that might come in contact with the blot. Cover the tray during incubation and washing steps. • Use non-powdered gloves, or switch to a different kind of gloves. We recommend powder-free nitrile gloves or polyethylene gloves. • Filter secondary antibody.

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