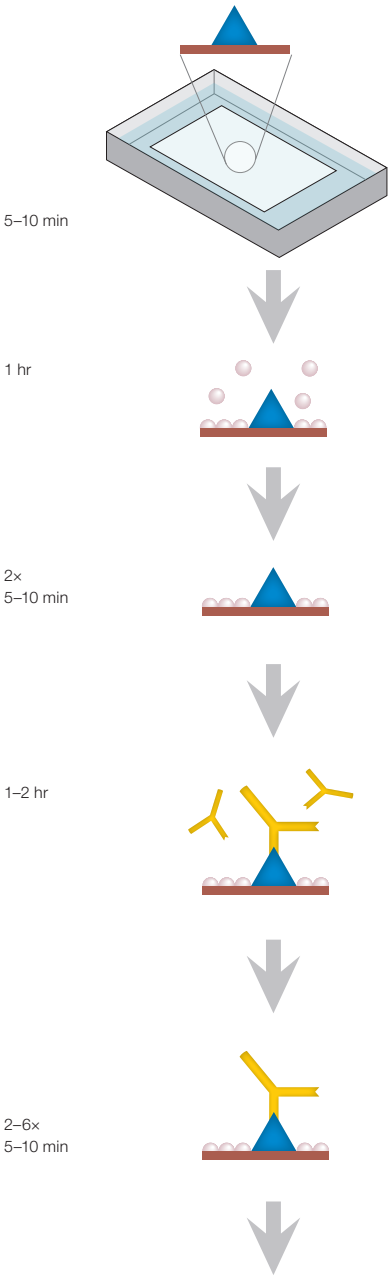
Immunodetection

Protocol

Bulletin 6219

Immunodetection



1 **Wash** — following transfer or protein application, wash the membrane for 5–10 min in TBS.

2 **Block** — incubate the membrane for 1 hr in blocking solution.

3 **Wash** — wash the membrane twice in TTBS, 5–10 min per wash.

4 **Primary antibody** — dilute the antibody in antibody dilution or blocking solution (refer to the instructions for the antibody for the recommended final concentration). Incubate the membrane for 1–2 hr in the primary antibody solution with gentle agitation.

5 **Wash** — wash the membrane 2–6 times in TTBS, 5–10 min per wash.

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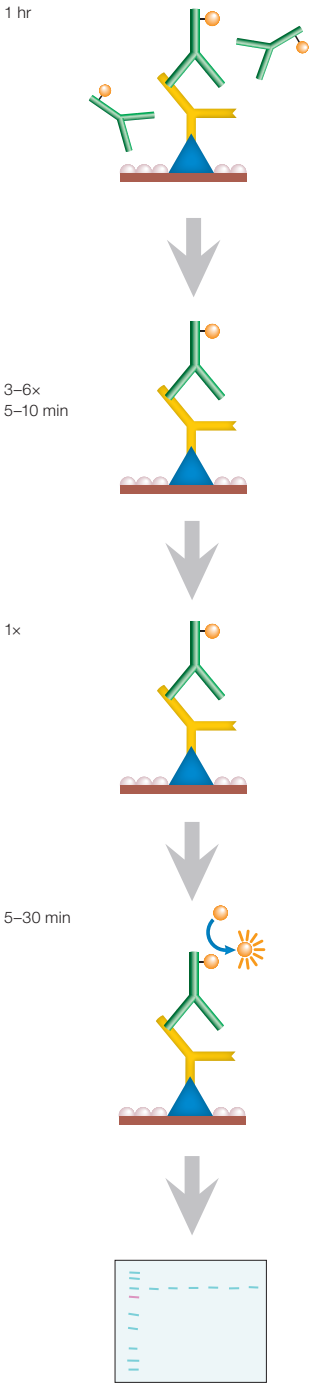

6 **Antibody conjugate** — dilute the conjugate in TTBS (refer to the instructions for the conjugate for the recommended final concentration). Incubate the membrane for 1 hr in the enzyme conjugate solution with gentle agitation.

7 **Wash** — wash the membrane 3–6 times in TTBS, 5–10 min per wash.

8 **Final wash** — wash the membrane in TBS to remove the Tween 20 from the membrane surface prior to blot development and imaging.

9 **Signal development** — for colorimetric development, add detection substrate and incubate for 5–30 min depending on the specific reagents used. For chemiluminescence/fluorescence, see next page.

10 **Image, dry, and store** — image the blot on a CCD laser-based imager, or expose to X-ray film or instant photographic film. Develop the film according to the manufacturer's instructions.

Notes for Multiplex Detection

Gel equilibration removes contaminating electrophoresis buffer salts. If not removed, these salts increase the conductivity of the transfer buffer and the amount of heat generated during transfer.

Equilibration also allows the gel to adjust to its final size prior to electrophoretic transfer. Gels shrink or swell to various degrees in the transfer buffer depending on the acrylamide percentage and the buffer composition.

Equilibration is not necessary (i) when the same buffer is used for both electrophoresis and transfer (for example, native gel transfers), or (ii) when using rapid semi-dry transfer systems such as the Trans-Blot® Turbo™ system (consult the user manual for the system you are using).

Notes for Chemiluminescence Detection

Follow steps 1–8 of the immunodetection assay, except use more stringent washes (steps 5 and 7). Wash the membrane six times for 10 min each at these steps, with strong agitation and a large volume of buffer to reduce background. Then follow below for step 9:

A. Place the membrane protein-side up on a clean piece of plastic wrap or a plastic sheet protector.

B. Add chemiluminescent substrate solution. Use at least 0.1 ml per cm^2 of membrane (about 6 ml for a standard 7 × 8.5 cm gel).

C. Incubate the membrane for 3–5 min in the chemiluminescent substrate solution.

D. Drain excess liquid from the blot and seal the membrane in a bag or sheet protector.

E. Image the blot on a CCD imager such as a ChemiDoc™ or VersaDoc™ system, or expose to X-ray film (for example, Kodak XAR or BioMax) or instant photographic film, such as Polaroid Type 667 or 612. Typical exposure times are 30 sec to 5 min. Develop the film according to the manufacturer's instructions.

Notes for Fluorescence Detection

Follow steps 1–8 of the immunodetection assay. Imaging of most fluorescent dye conjugates (Cy, Dylight, Alexa Fluor, and IRDye dyes) can be performed on wet or dry membranes. Imaging of fluorescent protein conjugates (phycoerythrin, allophycocyanin) should be performed on wet membranes for maximum sensitivity. Refer to the table below for recommended imager settings. Excitation and emission wavelengths are similar for non-Bio-Rad imagers as well.

	Red excitation (e.g., Alexa 647, Cy5, DyLight 649)	**Blue excitation** (e.g., FITC, Alexa 488, DyLight 488)	**Green excitation** (e.g., Alexa 555, Cy3, DyLight 548, TAMRA)
VersaDoc MP PharosFX™	695 BP 635 Ex/695 BP	530 BP 488 Ex/530 BP	605 BP 532 Ex/605 BP

Note for Protein G-HRP Detection

Follow steps 1–4 on previous page. For step 5 (wash), use TCBS instead of TTBS and then continue with steps 6–10.

Notes for Amplified Opti-4CN™ Detection

Follow steps 1–8 of the immunological assay on previous page. Then:

A. Incubate the membrane in diluted BAR for 10 min.

B. Wash the membrane 2–4 times in 20% DMSO/PBST for 5 min each time.

C. Wash 1–2 times in PBST for 5 min. each time.

D. Incubate the membrane and diluted streptavidin-HRP for 30 min.

E. Wash the membrane twice in PBST for 5 min each time.

F. Continue with steps 9–10.

Notes for Amplified AP Detection

Follow steps 1–5 of the immunodetection assay on previous page. Then:

A. Incubate the membrane for 1–2 hr in biotinylated secondary antibody solution.

B. While the blot is incubating in the biotinylated antibody solution, prepare the streptavidin-biotinylated AP complex. Allow the complex to form for 1 hr at room temperature.

C. Wash the membrane twice in TTBS, 5–10 min per wash.

D. Incubate the membrane for 1–2 hr in the streptavidin complex solution.

E. Continue with steps 7–10.

TIPS

If kept wet, blots using HRP or AP conjugates can be stored for several days prior to development and imaging. Leave blot in TBS, or place membrane between two pieces of filter paper soaked in TBS, and place in a sealable container.

This is an excerpt from Bio-Rad's comprehensive Protein Blotting Guide (Bulletin 2895).

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