

WORKFLOW

CLICK LINKS:

Membrane Preparation

Electrophoretically transfer separated proteins onto PVDF or nitrocellulose membrane

Protran™ Nitrocellulose Transfer Membranes

0.2 µm

0.45 µm

Blotting Paper

Membrane Blocking

Block nonspecific binding sites by incubating the membrane in the blocking buffer and wash

Bovine Serum Albumin (BSA)



Antibody Incubation

- Incubate the membrane with the primary antibody and wash
- Incubate the membrane with the secondary antibody and wash

Secondary antibodies & Proteins

Horseradish peroxidase (HRP) conjugates

Alkaline phosphatase (AP) conjugates

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Chemiluminescent substrates

- Western Lightning™
- Western Lightning™ ONE

Detection & Visualization

Incubate the membrane with appropriate substrate and prepare blot for imaging

- For chemiluminescence substrates, detect proteins using imager or film
- See chromogenic blots with the naked eye or imaging device



Chromogenic substrates

Stripping & Reprobing

(optional: for chemiluminescent detection only)

- Incubate with stripping buffer and wash
- Incubate with chemiluminescent substrate
- Expose to film or imager to make sure the original signal is removed
- Go back to the blocking step



Additional information

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Look also:
[Western Blotting Solutions Brochure](#)