



FILTER TECHNOLOGY

WESTERN BLOT GENERAL PROTOCOL



Western Blot General Protocol

Main Solutions and Reagents for running, transfer and blocking

Running buffer 10X:

- Tris base: 250 mM
- Glycine: 1.90 M
- SDS: 1%.

The pH of the buffer should be 8.3 and no pH adjustment is required. Store the running buffer at room temperature and dilute before use.

Running buffer 1X:

- 10% 10X Running buffer
- 90% DW H₂O

Tris Glycine Buffer 1X:

- 25 mM Tris base
- 190 mM Glycine

Transfer Buffer:

- 20% MetOH
- 0.25X Tris Glycine buffer

Phosphate Buffered Saline (PBS) 1X:

- 137 mM NaCl
- 2.7 mM KCl
- 10 mM Na₂HPO₄
- 1.8 mM KH₂PO₄

PBS Tween (PBST) 1X:

- 0.05% Tween
- 99.95% PBS 1X

Blocking Buffer:

- 5% skim milk (or Bovine Serum Albumin - BSA)
- 95% PBST

Blocking and antibody incubation

- Incubate membrane for 1h in the blocking buffer at room temperature or overnight at 4°C with constant agitation.
The active side of the membrane must always be in contact with the solution.
- Place the blot in the primary antibody solution and incubate with agitation for 1 hour at room temperature.
The solution should move freely across the surface of the membrane (dilution of the antibody depends on the producer recommendation).
- Wash membrane by:
 - Immersion in PBS-Tween (PBST) for 10 minutes with agitation;
 - Immersion in PBS-Tween (PBST) for 5 minutes with agitation (2 times).
- Place the blot in the secondary antibody solution (HRP conjugates) and incubate with agitation for 45 minutes at room temperature.
Dilution of the antibody depends on the producer recommendation.
- Wash the membrane according to the washing steps described in point 3 of Blocking and antibody incubation section.

Detection via chemiluminescence

- Prepare a 1:1 mixture of chemiluminescent substrate (ECL HRP, depending on sensitivity choose Light Wave; Light Wave Plus or Light Wave Max) .
- Place the blot in the container with substrate and incubate for 3 minutes.
- Remove the excess of the solution off the membrane.
- Place membrane in blot development folder and gently smooth out all the bubbles using a roller.
- Expose the film to the imaging system.

PROCEDURE

Electrophoresis – protein separation

- Prepare appropriate SDS-Polyacrylamide (SDS-PAGE) gel for electrophoresis.
Type of SDS-PAGE gels according to the protein size; the lower is the protein size, the higher concentration of gel should be used.
- Prepare the sample to be loaded in the wells of SDS-PAGE gel.
Preparation of the sample and the sample buffer depends on the type of the protein and manufacturer's recommendations.
- Load protein marker and equal volumes of protein sample into corresponding wells of SDS-PAGE gel.
Fill the empty wells with the sample buffer.
- Fill the electrophoresis tank with running buffer.
- Run the gel in following conditions:
 - 120 V for 20-30 minutes (or until the sample reaches the stacking gel);
 - 180 V for 30-45 minutes (separation of the proteins under constant voltage).

Electrotransfer of Proteins

- In case of PVDF membrane perform membrane equilibration by:
 - Immersing membrane in Methanol for 1 minute;
 - Followed by immersion of membrane in DW water for 5 minutes;
 - Followed by immersion of membrane in Transfer Buffer for 10 minutes.*Membrane must be wet at all times.*
- Assemble the transfer sandwich according to scheme presented in Figure 1.
Ensure there are no bubbles between the gel and the membrane.
- Place the cassette in the transfer tank and fill the Electroblotting tank with the transfer buffer (ensure that the sandwich is covered with the buffer).
- Run the Electroblotting for 1 hour at 120 V in an ice bath.
Running conditions might need optimization.

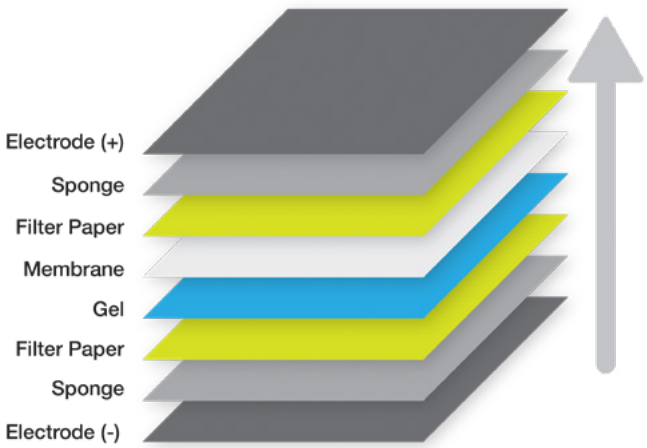
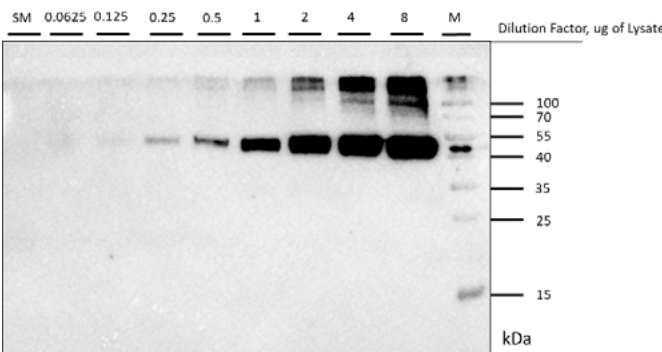


Figure 1 Scheme of transfer sandwich assembly.

GVS 0.45 µm PVDF Transfer Membrane



Images were obtained by following GVS Western Blot General Protocol

Cell Lane: HeLa Whole Cell

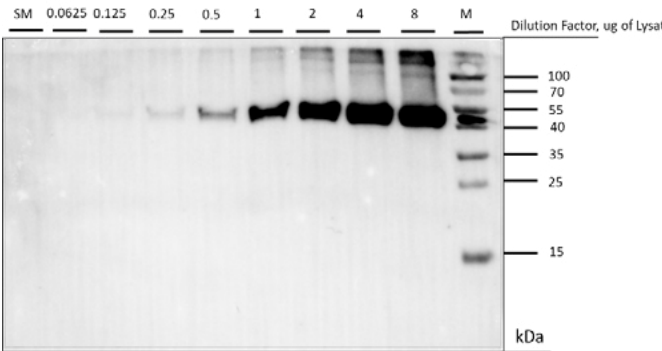
Detection substrate: Light Wave Plus

Primary antibody:
Beta Actin Polyclonal Antibody (dilution 1:1000)

Secondary antibody:
Goat Anti-Rabbit IgG Antibody (H+L) (dilution 1:10000)

Analyzed protein: Beta actin, MW: 42 kDa

Competitor 0.45 µm PVDF Transfer Membrane





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