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

PROCEDURE

Electrophoresis – protein separation

1. 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.
2. 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.
3. Load protein marker and equal volumes of protein sample into corresponding wells of SDS-PAGE gel.
   Fill the empty wells with the sample buffer.
4. Fill the electrophoresis tank with running buffer.
5. Run the gel in following conditions:
   a. 120 V for 20-30 minutes (or until the sample reaches the stacking gel);
   b. 180 V for 30-45 minutes (separation of the proteins under constant voltage).

Electrotransfer of Proteins

1. In case of PVDF membrane perform membrane equilibration by:
   a. Immersing membrane in Methanol for 1 minute;
   b. Followed by immersion of membrane in DW water for 5 minutes;
   c. Followed by immersion of membrane in Transfer Buffer for 10 minutes.

   Membrane must be wet at all times.
2. Assemble the transfer sandwich according to scheme presented in Figure 1.
   Ensure there are no bubbles between the gel and the membrane.
3. 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).
4. Run the Electroblotting for 1 hour at 120 V in an ice bath.
   Running conditions might need optimization.

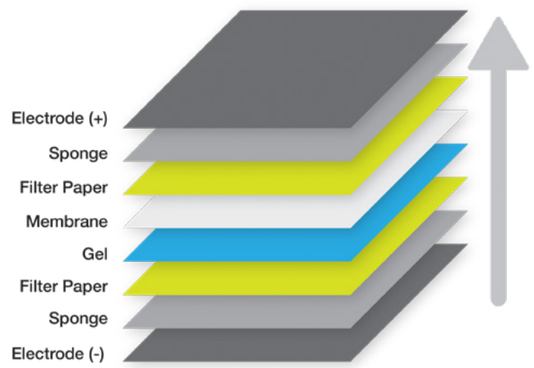


Figure 1 Scheme of transfer sandwich assembly.

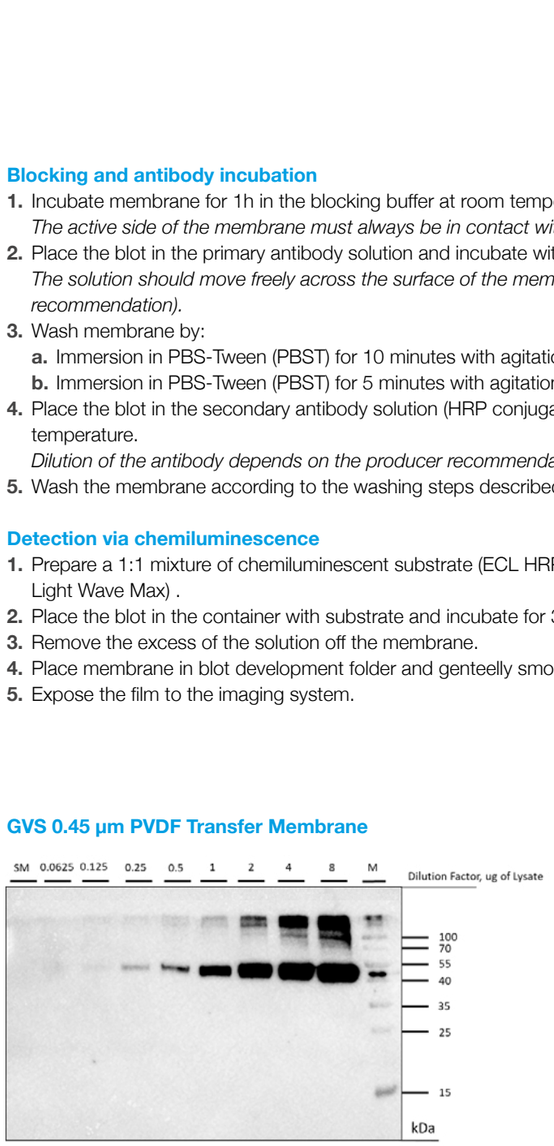
Blocking and antibody incubation

1. 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.
2. 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).
3. Wash membrane by:
   a. Immersion in PBS-Tween (PBST) for 10 minutes with agitation;
   b. Immersion in PBS-Tween (PBST) for 5 minutes with agitation (2 times).
4. 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.
5. Wash the membrane according to the washing steps described in point 3 of Blocking and antibody incubation section.

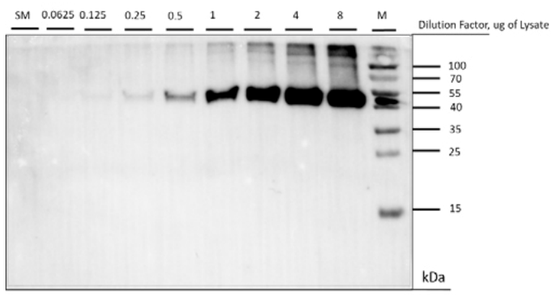
Detection via chemiluminescence

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

GVS 0.45 µm PVDF Transfer Membrane



Competitor 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

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