



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 H<sub>2</sub>O

### 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 Na<sub>2</sub>HPO<sub>4</sub>
- 1.8 mM KH<sub>2</sub>PO<sub>4</sub>

### 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.*

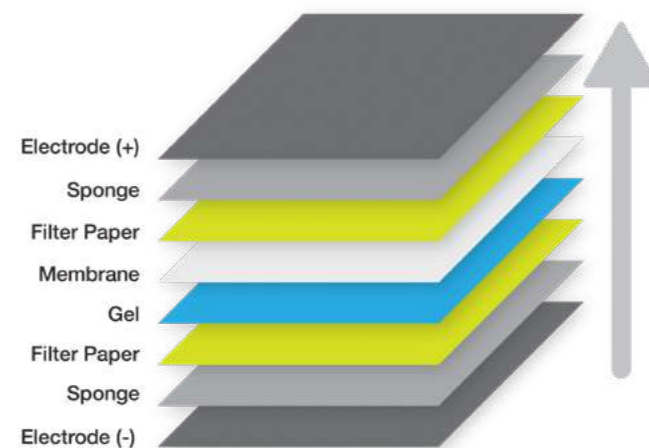
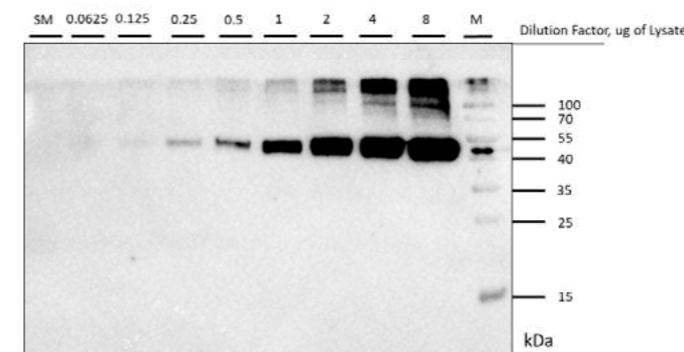


Figure 1 Scheme of transfer sandwich assembly.

### GVS 0.45 µm PVDF Transfer Membrane



Images were obtained by following GVS Western Blot General Protocol

Cell Lane: HeLa Whole Cell

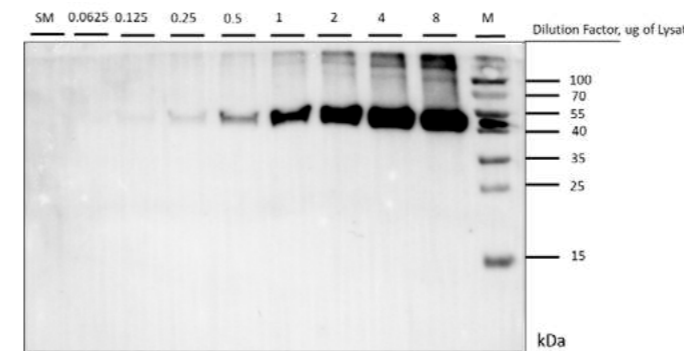
Detection substrate: Light Wave Plus

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





**WORLDWIDE**  
DISTRIBUTION CENTERS



**EUROPE**

**Italy Office**  
Headquarters  
GVS S.p.A.  
Via Roma 50  
40069 Zola Predosa (BO) - Italy  
Tel. +39 051 6176311  
Fax +39 051 6176200  
lifesciences.it@gvs.com

**United Kingdom**  
GVS Filter Technology UK Ltd.  
NFC House  
Vickers Industrial Estate  
Mellishaw Lane, Morecambe  
Lancashire LA3 3EN  
Tel. +44 (0) 1524 847600  
lifesciences.uk@gvs.com

**Russia**  
GVS Russia LLC  
Profsoyuznaya Street, 25-A, office 102  
117418, Moscow  
Russian Federation (Russia)  
Tel. +7 495 0045077  
lifesciences.ru@gvs.com

**Romania**  
GVS Microfiltrazione srl  
Sat Ciorani de Sus 1E  
107156 Ciorani  
Prahova România  
Tel. +40 244 463044  
lifesciences.ro@gvs.com

**Turkey**  
GVS Türkiye  
Cevizli mah. Zuhul cad. Ritim Istanbul  
no: 44 A-1 Blok D.371 Maltepe / Istanbul  
Tel. +90 216 504 47 67  
lifesciences.tr@gvs.com

**ASIA**

**China**  
GVS Technology (Suzhou) Co., Ltd.  
Fengqiao Civil-Run Sci-Tech Park,  
602 Changjiang Road, S.N.D.  
Suzhou, China 215129  
Tel. +86 512 6661 9880  
Fax: +86 512 6661 9882  
lifesciences.cn@gvs.com

**Japan**  
GVS Japan K.K.  
KKD Building 4F, 7-10-12 Nishishinjuku  
Shinjuku-ku, Tokyo 160-0023 Japan  
Tel. +81 3 5937 1447  
Fax +81 3 5937 1448  
lifesciences.jp@gvs.com

**Korea**  
GVS Korea Ltd #315 Bricks Tower  
368 Gyungchun-ro(Gaun-dong),  
472060 Namyangju-si, Gyunggi-do  
Tel: +82 31 563 9873  
Fax: +82 31 563 9874  
lifesciences.kr@gvs.com

**India**  
GVS Filter India Pvt Ltd  
Unit No 35 & 36 on First Floor  
Ratna Jyot Industrial Premises Irla Lane,  
Irla Vile Parle, Mumbai 400056, India  
lifesciences.in@gvs.com

**Malaysia**  
GVS Filtration Sdn.Bhd  
Lot No 10F-2B, 10th Floor, Tower 5 @ PFCC  
Jalan Puteri 1/2, Bandar Puteri  
47100 Puchong, Selangor, Malaysia  
Tel: +60 3 7800 0062  
lifesciences.my@gvs.com

**Thailand**  
GVS Filtration Co., Ltd.  
88 Ratchadaphisek Rd,  
Office 10E03 - Khlong Toei,  
Bangkok 10110  
lifesciences.th@gvs.com

**AMERICA**

**U.S.A.**  
GVS North America, Inc.  
63 Community Drive  
Sanford, ME 04073 - USA  
Tel. +1 866 7361250  
lifesciences.us@gvs.com

**Puerto Rico**  
GVS Puerto Rico, LLC  
98 Carr 194 - Fajardo,  
Puerto Rico, 00738-2988, USA  
Tel. +1.787.355.4100  
e-mail: gvspuertorico@gvs.com

**Mexico**  
GVS de México  
Universal No. 550, Vynmsa Aeropuerto Apodaca  
Industrial Park, Ciudad Apodaca, Nuevo León,  
C.P. 66626 México  
Tel. +52 81 2282 9003  
lifesciences.mx@gvs.com

**Brazil**  
GVS do Brasil Ltda.  
Rodovia Conego Cyriaco Scaranello Pires 251  
Jardim Chapadão, CEP 13193-580  
Monte Mor (SP) - Brasil  
Tel. +55 19 38797200  
Fax +55 19 38797251  
lifesciences.br@gvs.com

**Argentina**  
GVS Argentina S.A.  
Francisco Acuña de Figueroa  
719 Piso:11 Of: 57  
1416 Buenos Aires - Argentina  
Tel. + 5411 48614750  
lifesciences.ar@gvs.com