



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

- 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.

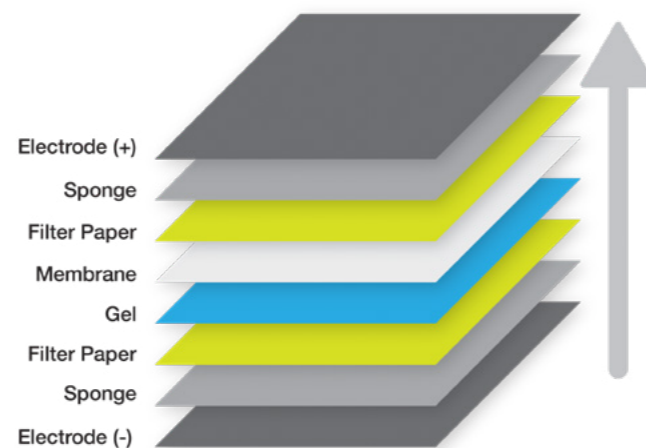


Figure 1 Scheme of transfer sandwich assembly.

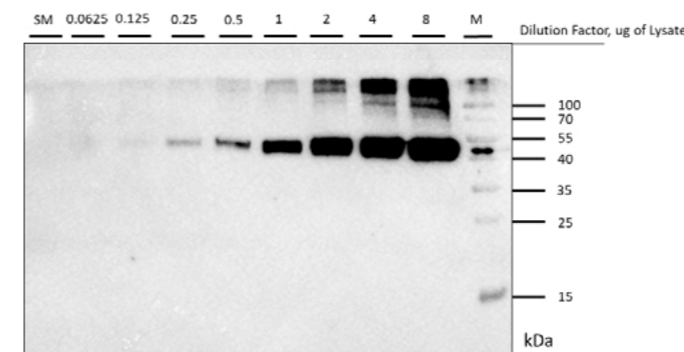
Blocking and antibody incubation

- 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.

GVS 0.45 µm PVDF Transfer Membrane



Images were obtained by following GVS Western Blot General Protocol

Cell Lane: HeLa Whole Cell

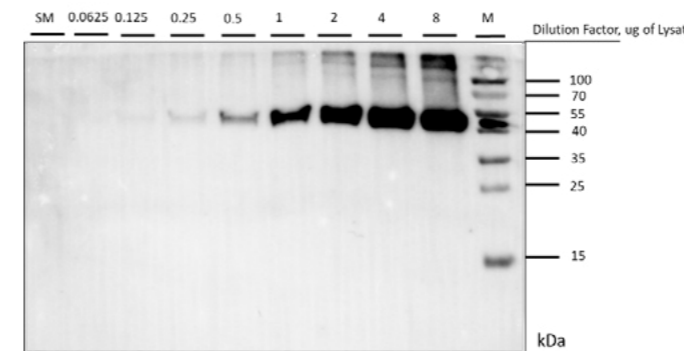
Detection substrate: Light Wave Plus

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

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

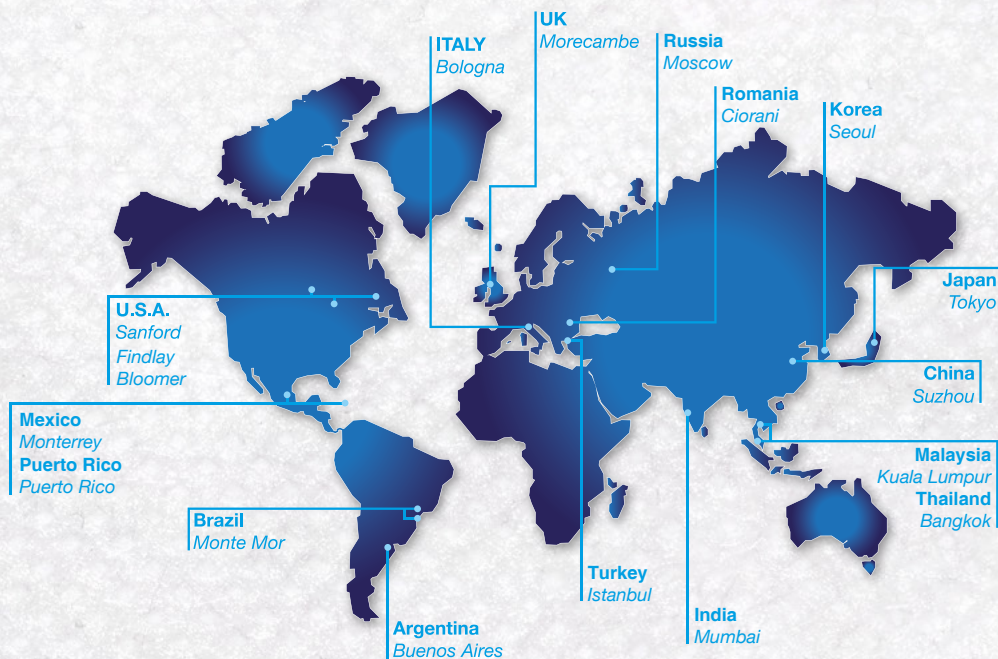
Analyzed protein: Beta actin, MW: 42 kDa

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