

## H00055148-T01 Protocol

# Western Blot Protocol

### SECTION 1 - Equipments & Reagents

#### 1.1 Equipment(s)

- Shaker (TKB OS701)
- AutoChemi System (UVP)

#### 1.2 Blocking buffer (also Dilution buffer)

Weigh non-fat milk 5 g and dissolve in 100 mL 1X PBST (0.2%) to a final mixture of 5% non-fat milk/PBST (0.2%).

#### 1.3 (10X) PBST (phosphate buffer saline)

NaCl	(0.13 M x 10, Merck 6404)	75.9 g
NaH <sub>2</sub> PO <sub>4</sub> -H <sub>2</sub> O	(0.01 M x 10, Merck A429146 335)	13.8 g

Add 800 mL ddH<sub>2</sub>O. After the salts have dissolved, use NaOH liquid to adjust the solution to pH 7.0 and make the final dilute solution to 1,000 mL. The solution becomes 10X PBS. Dilute the solution with ddH<sub>2</sub>O to the final 1X PBS prior application.

#### 1.4 PBST (0.2%) (Phosphate buffer saline and 0.2% Tween 20)

1X PBS	1 L
Tween 20	2 mL

Dilute 10X PBS with ddH<sub>2</sub>O to a final 1X concentration. Add Tween 20 [0.2% (v/v)] to final PBST (0.2%).

#### 1.5 Anti-IgG Secondary antibody

Use one of the following secondary antibodies according to the species of primary antibody raised against (please refer to Table 2 for detail information):

- a. Goat Anti-Mouse IgG (H&L)-HRP Conjugate secondary antibody (Abnova, Cat. No. PAB0096)
- b. Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, INC., Catalog No. 115-035-062).
- c. Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated (Pierce, Cat. No. 31460)

#### 1.6 Chemiluminescent reagent

SuperSignal West Femto Maximum Sensitivity Substrate (PIERCE, Cat. No. 34094, 34095 & 34096)  
Reagent must be freshly prepared each time before application.

### SECTION 2 - Assay Protocol

Please follow Current Protocols on SDS-PAGE gel running and Western Transfer. (Refer to Table 1 for the effective range of separation of SDS-PAGE)

2.1 Add adequate amount of blocking buffer and incubate the membrane at room temperature for 1 hour or under 4C overnight.

\*Please keep the membranes at -20C no longer than a week if the membranes will not be used immediately.

2.2 Dilute the primary antibody with fresh blocking buffer to the designated concentration. Remove the membrane from the previous blocking buffer and add the diluted primary antibodies to the membrane. Incubate at 4C overnight.

\*Please refer to Table 2 for dilution factors.

2.3 Wash membrane with PBST (0.2%) for 10 minutes. Repeat 3 times.

2.4 Add adequate amount of anti-IgG secondary antibody (please refer to Table 2 for detail). Leave the membrane at room temperature for 1 hour.

2.5 Wash membrane with PBST (0.2%) for 10 minutes. Repeat 4 times.

2.6 After washing, place membrane into a sealable bag and add freshly prepared chemiluminescent reagents into the bag to coat the entire membrane.

\*For maximum sensitivity, PIERCE SuperSignal West Femto Maximum sensitivity substrate is recommended. The reagent should be in 1:1 dilution of reagent A and B as working solution (Strictly follow the provider's instructions). Add working solution to the membrane and seal the bag. Spread the chemiluminescent reagent around so it can be distributed evenly onto the membrane. (Chemiluminescent reagent must be spread out evenly otherwise parts of the membrane will be over-exposed when the photograph is taken.) 0.6 mL of working solution is used for membrane size 8 cm x 10 cm.

2.7 Take photographs immediately with CCD camera at 5-second, 20-second, 1.5-minute, and 5-minute intervals, in order to acquire proper exposure image.

\*Strong signals may intensify into blackout signals with hollow band. In this case, dilute the secondary antibody.

Table 1. Effective Range of Separation of SDS-PAGE

Acrylamide Concentration (%)	Linear Range of Separation (kDa)
15	10 - 43
12	12 - 60
10	20 - 80
8	36 - 94
6	57 - 212

Table 2. Recommended Antigen amount and Antibody dilution use in Western Blot.

Type of Antigen	Cell/Tissue	Lysate Mammalian overexpressed lysate	Purified Protein
Antigen	Loading amount	25 ~ 50 ug/lane 15 ul/lane	0.1 ~ 0.2 ug/lane
Abnova's Primary Antibody (dilution or concentration)			
Mouse polyclonal antibody	1:500 ~ 1:1000	1:500 ~ 1:1000	1:1000 ~ 1:2500
Mouse MaxPab	1:500 ~ 1:1000 (or 1 ug/ml for purified Mouse MaxPab)		
Rabbit MaxPab	1:1000 (or 1 ug/ml for purified Rabbit MaxPab)		
Hybridoma cell culture supernatant (Mouse Ig)		Undiluted ~ 1:5	
Ascites (Mouse Ig)		1:500 ~ 1:1000	
Monoclonal antibody (Mouse Ig)		1 ~ 5 ug/ml	
Secondary Antibody (dilutions or concentration)			
Goat Anti-Mouse IgG (Abnova)	1:2500 ~ 1:5000	1:2500 ~ 1:5000	1:5000 ~ 1:10000
Goat Anti-Mouse IgG (Jackson Immunoresearch)	1:5000 ~ 1:10000	1:5000 ~ 1:10000	1:10000 ~ 1:15000
Goat Anti-Rabbit IgG (Pierce)		1:7500	

Note:

- The information provide in this table shall be used only as a guide, each investigator should determine the optimal antigen amount and antibody dilution for their own specific research application.
- Goat Anti-Mouse IgG (H&L)-HRP Conjugate secondary antibody (Abnova Corp., Catalog No. PAB0096).
- Peroxidase AffiniPure Goat Anti-Mouse IgG (H+L) (Jackson Immunoresearch Laboratories, INC., Catalog No.115-035-062).
- When the primary antibody is raised in rabbit, please use Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated (Pierce, Cat. No. 31460)
- Please follow the instruction on the product sheet, if it has the recommend dilutions of the primary antibody.