

## General Detection Buffer

### Tris-buffered saline (TBS), 2 L

20 mM Tris-HCl, 500 mM NaCl (pH 7.5)  
(catalog #170-6435, 1 L, 10x)

Tris base	4.84 g
NaCl	58.48 g
diH <sub>2</sub> O	1.5 L

Adjust pH to 7.5 with HCl.

Adjust volume to 2 L with diH<sub>2</sub>O.

### TTBS wash solution, 1 L

20 mM Tris-HCl, 500 mM NaCl, 0.05% Tween 20 (pH 7.5)  
0.5 ml Tween 20  
1 L TBS

### Citrate-buffered saline (CBS)

20 mM citrate, 500 mM NaCl (pH 5.5)  
Included in Immun-Blot® protein G kits.

### TCBS wash solution, 1 L

20 mM citrate, 500 mM NaCl, 0.05% Tween 20 (pH 5.5)  
0.5 ml Tween 20  
1 L CBS

### Blocking solution, 100 ml

3% gelatin-TBS  
Add 3.0 g gelatin to 100 ml TBS.  
Heat to 50°C; stir to dissolve.

or

3% BSA-TBS  
Add 1.0 g BSA to 100 ml TBS; stir to dissolve.

or

5% nonfat milk-TBS  
Add 5.0 g nonfat dry milk to 100 ml TBS; stir to dissolve.

**Note:** Gelatin can clog membranes and cut off the vacuum flow of microfiltration units; use an alternative blocking solution with the Bio-Dot® or Bio-Dot SF apparatus.

**Note:** Nonfat milk is not recommended for avidin/biotin systems as milk contains endogenous biotin and may cross-react with avidin-containing components in the detection system.

### Antibody dilution buffer, 200 ml

1% gelatin-TTBS  
Add 2.0 g gelatin to 200 ml TTBS.  
Heat to 50°C; stir to dissolve.

or

3% BSA-TTBS  
Add 6.0 g BSA to 200 ml TTBS; stir to dissolve.

or

5% nonfat milk-TTBS  
Add 10.0 g nonfat dry milk to 200 ml TTBS; stir to dissolve.

**Note:** Gelatin can clog membranes and cut off the vacuum flow of microfiltration units; use an alternative blocking solution with the Bio-Dot® or Bio-Dot SF apparatus.

**Note:** Nonfat milk is not recommended for avidin/biotin systems as milk contains endogenous biotin and may cross-react with avidin-containing components in the detection system.

### Antibody buffer (for chemiluminescence, ImmunStar™ AP only)

0.2% nonfat milk-TTBS  
Add 0.4 g nonfat milk to 200 ml TTBS; stir to dissolve.

### Antibody buffer for protein G-HRP, 100 ml

1% gelatin-TCBS  
Add 1.0 g gelatin to 100 ml TCBS.  
Heat to 50°C; stir to dissolve.

### Protein G-HRP conjugate solution, 100 ml

Mix 33 µl protein G conjugate solution in 100 ml 1% gelatin in TCBS.

### Streptavidin-biotinylated AP complex, 100 ml

33 µl streptavidin  
100 ml TTBS  
33 µl biotinylated AP

Incubate the complex 1–3 hr at room temperature before use.

## Total Protein Staining Buffers and Solutions

### Amido black staining solution, 1 L

For nitrocellulose:	
Amido black	5 g
Methanol	400 ml

Adjust volume to 1 L with diH<sub>2</sub>O.

or

Amido black	5 g
Isopropanol	250 ml
Acetic acid	100 ml

Adjust volume to 1 L with diH<sub>2</sub>O.

For PVDF:	
Amido black	1 g
Methanol	400 ml
Acetic acid	100 ml

Adjust volume to 1 L with diH<sub>2</sub>O.

### Amido black destain solution, 1 L

For nitrocellulose:	
Isopropanol	250 ml
Acetic acid	100 ml

Adjust volume to 1 L with diH<sub>2</sub>O.

For PVDF:	
Methanol	400 ml
Acetic acid	100 ml

Adjust volume to 1 L with diH<sub>2</sub>O.

### Coomassie Blue R-250 staining solution, 1 L

Coomassie Blue R-250	1 g
Methanol	400 ml
Acetic acid	100 ml

Adjust volume to 1 L with diH<sub>2</sub>O.

### Coomassie Blue R-250 destaining solution, 1 L

Methanol	400 ml
Acetic acid	100 ml

Adjust volume to 1 L with diH<sub>2</sub>O.

### Ponceau S staining solution

Ponceau S	2 g
Trichloroacetic acid (TCA)	30 g
Sulfosalicylic acid	30 g
diH <sub>2</sub> O	80 ml

### Ponceau S destaining solution

1% acetic acid or PBS

### SYPRO Ruby blot pretreatment solution

Acetic acid	70 ml
Methanol	100 ml
diH <sub>2</sub> O	830 ml

### Colloidal gold blot staining solution

Use TTBS wash solution.

## Substrate Buffers and Solutions

### HRP Substrate Buffers

4-(chloro-1-naphthol)	4CN	60 mg
	Methanol	20 ml
Protect mixture from light.		
	3% H <sub>2</sub> O <sub>2</sub>	600 µl
	Substrate solution	100 ml

Mix the two solutions together.

Use immediately. Alternatively, use HRP conjugate substrate solution in kit format.

### HRP conjugate solution

Dissolve contents of premixed substrate color development buffer in diH<sub>2</sub>O to 1 L

Color reagent B	600 µl
Development buffer	100 ml
HRP color reagent A	20 ml

Use immediately.

### Diaminobenzidine (DAB)

DAB	50 mg
TBS	100 ml
3% H <sub>2</sub> O <sub>2</sub>	100 µl

Use immediately.

### AP Substrate Buffers

AP color development buffer	MgCl <sub>2</sub>	0.233 g
	Tris base	12.1 g
	diH <sub>2</sub> O	800 ml

Adjust pH to 9.5 with HCl; adjust volume to 1 L with diH<sub>2</sub>O.

### 5-bromo-4-chloroindolyl phosphate/nitroblue tetrazolium (BCIP/NBT)

Dimethylformamide	0.7 ml
diH <sub>2</sub> O	0.3 ml
NBT	30 mg
Dimethylformamide	1 ml
BCIP	15 mg

Add both solutions to 100 ml AP color development buffer. Use immediately. Alternatively, use AP conjugate substrate solution in kit format.

### Immun-Star™ AP substrate solution (kit format)

Use 5 ml chemiluminescent substrate per 100 cm<sup>2</sup>.

For nitrocellulose membrane blots: Add 500 µl enhancer reagent to 10 ml Immun-Star chemiluminescent substrate. Store at 4°C for up to 1 week.

For PVDF membrane blots: Immun-Star AP generates a very fast light signal on PVDF membrane; therefore, the use of an enhancer is not necessary. The substrate is provided ready to use.

### Immun-Star HRP substrate solution (kit format)

For nitrocellulose and PVDF membrane blots: A 1:1 mixture of luminol/ enhancer to peroxide buffer is recommended. Use 10 ml per 100 cm<sup>2</sup> of membrane (12 ml for one 8.5 × 13.5 cm Criterion™ blot).

## Stripping Buffer

### Acidic glycine stripping buffer

Glycine	7.5 g
Mg(CH <sub>3</sub> COO) <sub>2</sub> ·4H <sub>2</sub> O	4.3 g
KCl	3.7 g
diH <sub>2</sub> O	800 ml
Adjust pH to 2.2 with HCl.	
diH <sub>2</sub> O	to 1 L

This is an excerpt from Bio-Rad's comprehensive Protein Blotting Guide (Bulletin 2895).



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