Detection Buffer Formulations

Protocol

Bulletin 6216

General Detection Buffer

Tris-buffered saline (TBS), 2 L
20 mM Tris-HCl, 500 mM NaCl (pH 7.5)
(catalog #170-6435, 1 L, 10×)

Tris base	4.84 g
NaCl	58.48 g
diH_2O	1.5 L

Adjust pH to 7.5 with HCl.

Adjust volume to 2 L with diH_2O.

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.

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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_2O.

or

Amido black	5 g
Isopropanol	250 ml
Acetic acid	100 ml

Adjust volume to 1 L with diH_2O.

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

Adjust volume to 1 L with diH_2O.

Amido black destain solution, 1 L

For nitrocellulose:	
Isopropanol	250 ml
Acetic acid	100 ml

Adjust volume to 1 L with diH_2O.

For PVDF:	
Methanol	400 ml
Acetic acid	100 ml

Adjust volume to 1 L with diH_2O.

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_2O.

Coomassie Blue R-250 destaining solution, 1 L

Methanol	400 ml
Acetic acid	100 ml

Adjust volume to 1 L with diH_2O.

Ponceau S staining solution

Ponceau S	2 g
Trichloracetic acid (TCA)	30 g
Sulfosalicylic acid	30 g
diH_2O	80 ml

Ponceau S destaining solution

1% acetic acid or PBS

SYPRO Ruby blot pretreatment solution

Acetic acid	70 ml
Methanol	100 ml
diH_2O	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_2O_2	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_2O 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_2O_2	100 µl
	Use immediately.	

AP Substrate Buffers

AP color development buffer	$MgCl_2$	0.233 g
	Tris base	12.1 g
	diH_2O	800 ml
	Adjust pH to 9.5 with HCl; adjust volume to 1 L with diH_2O.	
5-bromo-4-chloroindolyl phosphate/nitroblue tetrazolium (BCIP/NBT)	Dimethylformamide	0.7 ml
	diH_2O	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^2.

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^2 of membrane (12 ml for one 8.5 × 13.5 cm Criterion™ blot).

Stripping Buffer

Acidic glycine stripping buffer

Glycine	7.5 g
$Mg(CH_3COO)_2 \cdot 4H_2O$	4.3 g
KCl	3.7 g
diH_2O	800 ml
Adjust pH to 2.2 with HCl.	
diH_2O	to 1 L

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

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