

High-performance Blocking Reagents

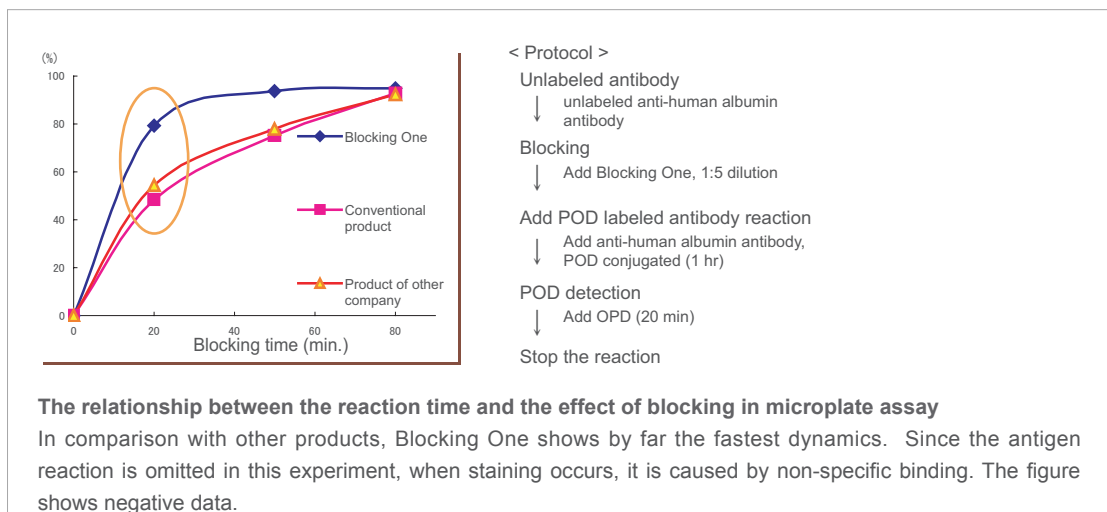
Blocking One Series

■ Blocking One

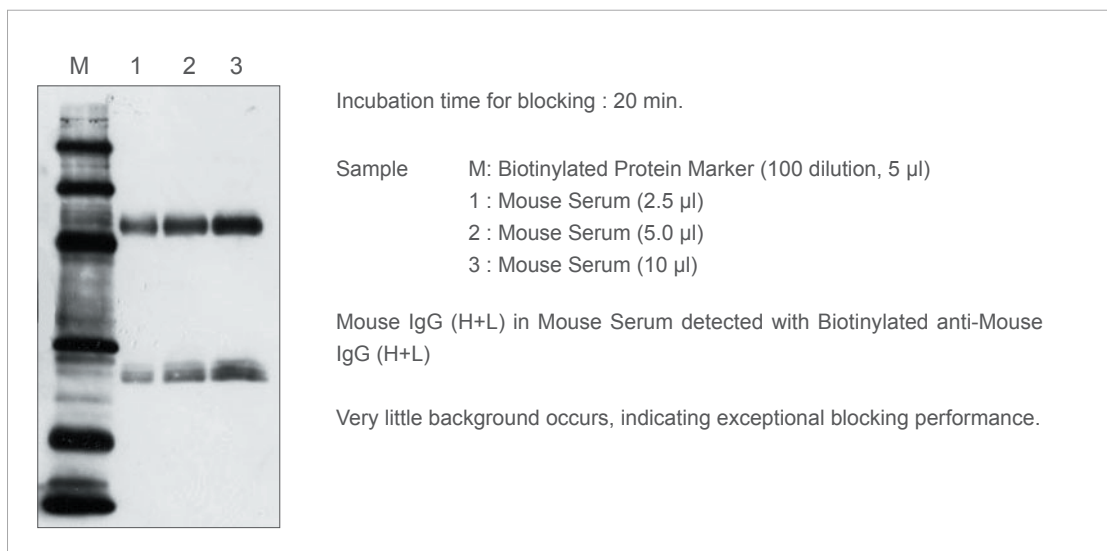
Blocking is indispensable in immunoassays in order to block non-specific binding reactions. Blocking One contains both high molecular weight compounds and bovine serum protein. Blocking One is superior to conventional blocking solutions. The preservative in Blocking One does not affect the enzyme activity of peroxidase (POD) or alkaline phosphatase (ALP). Only simple refrigerator storage is necessary, even after opening the bottle.

- » **Fast** : in many assays a reduction of incubation time for blocking can be realized
- » **Easy to use** : simple storage in refrigerator even after opening the bottle

Comparison of blocking efficiency



Detection of Mouse IgG(H+L) by Western Blotting



■ Blocking One-P

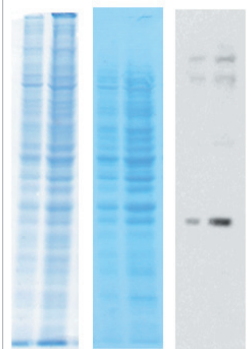
In immunological detection methods, blocking agents containing phosphoproteins such as skim milk are not well suited for immunoassays with phospho-specific antibodies, and cause high background. Blocking One-P is an exclusive blocking solution, free of phosphate group and endogenous phosphatase. For phospho protein detection. The performance is superior compared with conventional blocking solutions such as 1% BSA. The preservative does not affect the enzyme activity of peroxidase (POD) or alkaline phosphatase (ALP). Only simple refrigerator storage is necessary, even after opening the bottle.

- » **Safe** : does not contain phosphorylated proteins
- » **Confirmed** : endogeneous phosphatase free
- » **High efficiency** : more effective than 1% BSA

Comparison

	Composition	Phospho-specific antibody applications	Blocking efficacy
Blocking One-P	- High molecular weight compounds - BSA	+++	+++
Blocking One	- High molecular weight compounds - BSA - Casein	+	+++
Skim milk	- Casein	-	+
1% BSA	- BSA	++	+

Western Blotting with phospho-specific antibody

CBB Staining	WB	Sample	
1 2 3 4	5 6		
		HeLa whole cell lysate (1.0×10^7) + RIPA Buffer (Product No.: 08714-04) (1 ml)	
		Lane 1. 5 μ l (SDS-PAGE Gel)	
		Lane 2. 10 μ l (SDS-PAGE Gel)	
		Lane 3. 5 μ l (Blotting Membrane)	
		Lane 4. 10 μ l (Blotting Membrane)	
		Lane 5. 5 μ l	
		Lane 6. 10 μ l	
		Blocking	Blocking One-P, 20 min.
		Primary antibody	Anti-p-Tyr(PY20)(Mouse), Monoclonal (Santa Cruz: #sc-508), 1:2000 dilution
		Secondary antibody	Anti-Mouse IgG-HRP (Santa Cruz: #sc-2005), 1:10000 dilution
		Detection	CBB Stain One (Product No.: 04543-51) Chemi-Lumi One L (Product No.: 07880-70)

Protocol (for Blocking One and Blocking One-P)

- For blocking in 96-well microplate

Apply 350 μ l prepared blocking solution with a micropipette into each well of the microplate and let it stand for 45 minutes at room temperature. Remove the blocking solution from the wells by turning the micro plate upside-down. Apply 350 μ l tTBS into the wells of the microplate to rinse out the blocking solution. Repeat 3 times. The plate is ready for antigen-antibody reaction.

For phospho-specific antibodies using Blocking One-P, we recommend adding phosphatase inhibitor (ex. Phosphatase Inhibitor Cocktail, Product No.: 07574-61) to inhibit dephosphorylation of the sample.

- For diluting enzyme labeled antibody

Add the enzyme labeled antibody of predetermined quantity into blocking solution diluted 20 times. Shake carefully not to generate foam, and use it immediately. Activity of the enzyme labeled antibody after dilution or the sensitivity of ELISA test may decrease over time. For phospho-specific antibodies using Blocking One-P, we recommend adding phosphatase inhibitor (ex. Phosphatase Inhibitor Cocktail, Product No.: 07574-61) to inhibit dephosphorylation of the sample.

- For blocking immunoblotting membrane

For a membrane (approx. 10 cm x 10 cm), pour 50 ml blocking reagent into a clean plastic tray. Immerse the membrane into the solution and shake for approx. 30 minutes (for Blocking One) and approx. 20 minutes (for Blocking One-P).

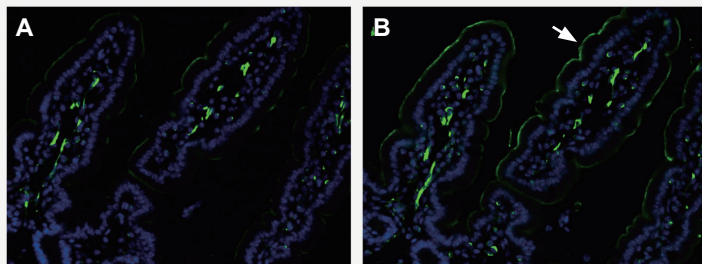
■ Blocking One Histo

Blocking One Histo is a blocking buffer to prevent non-specific binding of antibodies in immunohistochemistry (IHC). The product is designed for immunohistochemistry application based on Blocking One.

- » **Easy to use** : eye-drop bottle
- » **Confirmed** : can be used for immunofluorescence staining
- » **Safe** : the preservative does not affect the activity of alkaline phosphatase or horseradish peroxidase



Comparison of blocking efficient with 10% Goat Serum (Immunofluorescence)



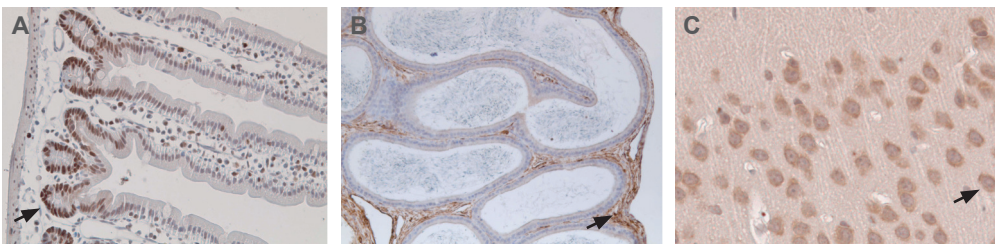
Blocking One Histo (10 min.)

10% Goat Serum (10 min.)

In both panels, mouse small intestine tissue section was stained with secondary antibody conjugated with CF™ 488A (green) and counter stained with DAPI (blue). In the panel B with 10% Goat Serum, the stained white arrow along the lines of shape of small intestine show non-specific staining. Blocking One Histo is more effective at reducing non-specific background staining than normal serum.

Sample	Mouse small intestine (Paraffin-embedded section)
Antigen Retrieval	Histo VT One, 90°C, 20 min.
Primary antibody	Anti-Vimentin rabbit polyclonal antibody (Santa Cruz: #sc-7557R)
Secondary antibody	CF™ 488A Goat anti-Rabbit IgG(H+L), F(ab') ₂ Fragment (Biotium: #20013)

Application data



A: Mouse small intestine (PCNA) x5
B: Mouse epididymis (Vimentin) x25
C: Mouse brain (GluR) x100

Antigen Retrieval	Histo VT One, Room temp., 10 min.
Primary antibody	A: Anti-PCNA rabbit pAb (Santa Cruz: #sc-7907) B: Anti-Vimentin rabbit pAb (Santa Cruz: #sc-7557R) C: Anti-GluR-1 goat pAb (Santa Cruz: #sc-7608)
Secondary antibody	A: Goat anti-rabbit IgG (H+L), biotin conjugated (Vector, #BA-1000) B: Goat anti-rabbit IgG (H+L), biotin conjugated (Vector, #BA-1000) C: Bovine anti-goat IgG (H+L), biotin conjugated (Santa Cruz: #sc-2347)
Detection	Streptavidin Biotin Complex Peroxidase Kit (Product No.: 30462) Peroxidase Stain DAB Kit (Brown Stain) (Product No.: 25985)

Blocking treatment of each tissue section had been performed by Blocking One Histo. Mouse small intestine (panel A) was stained with anti-PCNA and DAB (3,3'-Diamino Benzidine) to stain nuclear (black arrow), Mouse epididymis (panel B) was stained with anti-Vimentin and DAB to stain muscle (black arrow), Mouse brain (panel C) was stained with anti-GluR and DAB to stain membrane proteins (black arrow) and counter stained with hematoxylin.

Protocol

- Blocking non-specific binding in IHC

Cover a tissue section on a slide glass with enough drops of this product. Incubate tissue section at room temperature for 5-10 minutes. Tap off blocking solution and wash slide glass with tPBS or tTBS for 5 minutes before application of primary antibody.

- Dilution antibody

Add predetermined quantity of antibody into the diluted blocking buffer. Shake gently not to generate foam. Use it immediately. Do not store diluted antibody solution because activity of enzyme labeled antibody after dilution may decrease over time.

Ordering Information

Product Name	Storage	Product Number	PKG Size
Blocking One	0-10°C	03953-95	500 ml
Blocking One-P	0-10°C	05999-84	200 ml
Blocking One Histo	0-10°C	06349-64	50 ml

Product Name	Storage	Product Number	PKG Size
Streptavidin Biotin Complex Peroxidase Kit	0-10°C	30462-30	1 Kit
Chemi-Lumi One Super	0-10°C	02230-30	1 Kit
Chemi-Lumi One L	0-10°C	07880-70	1 Kit
WB Stripping Solution	0-10°C	05364-55	500 ml
WB Stripping Solution Strong	0-10°C	05677-65	500 ml
Peroxidase Stain DAB Kit (Brown Stain)	0-10°C	25985-50	1 Kit
Peroxidase Stain DAB Kit (Dark-gray Stain)	0-10°C	25986-40	1 Kit
Peroxidase Stain Kit for Immuno-blotting, Nuclease tested	-20°C	26652-70	1 Kit
BCIP-NBT Solution Kit for Alkaline Phosphatase Stain, Nuclease tested	-20°C	03937-60	1 Kit
ELISA POD Substrate A. B. T. S. Kit	0-10°C	14351-80	1 Kit
ELISA POD Substrate TMB Kit (Popular)	0-10°C (Do not Freeze)	05298-80	1 Kit
ELISA POD Substrate TMB Solution (Easy)	0-10°C (Do not Freeze)	05299-54	100 ml

For research use only, not intended for diagnostic or drug use.

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