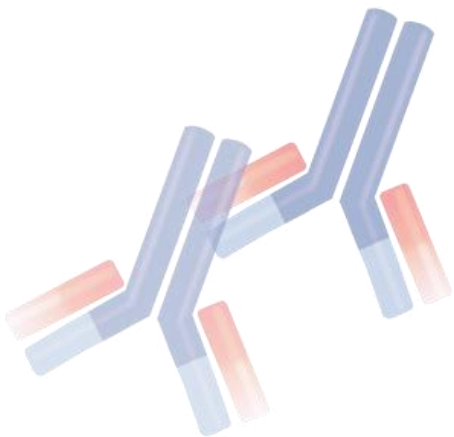
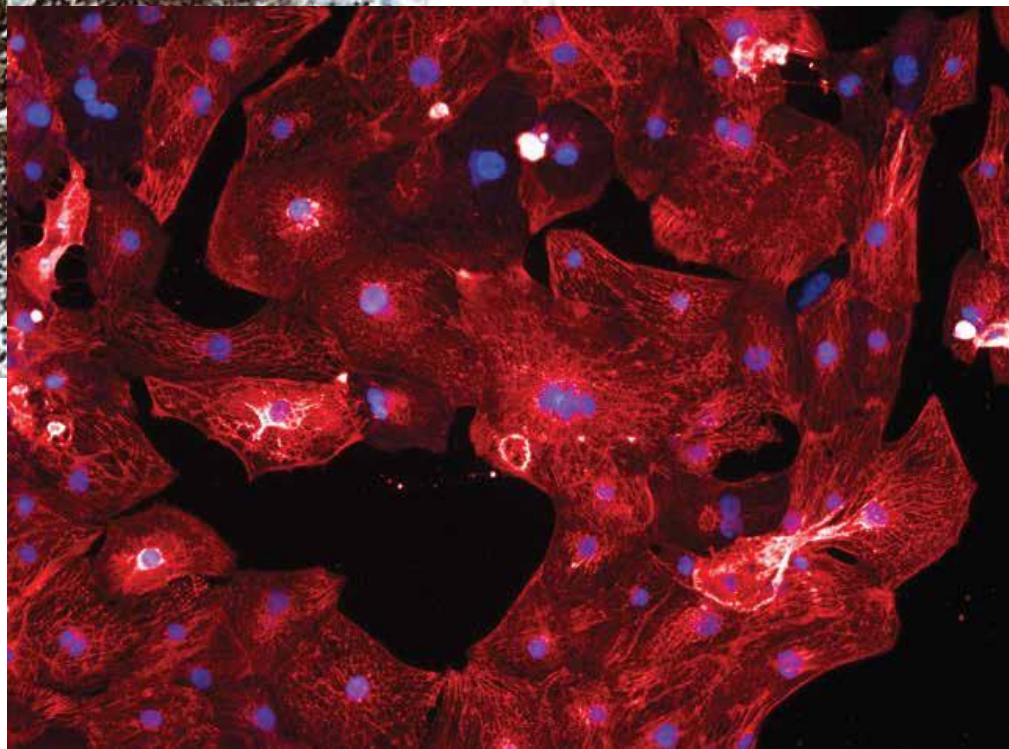
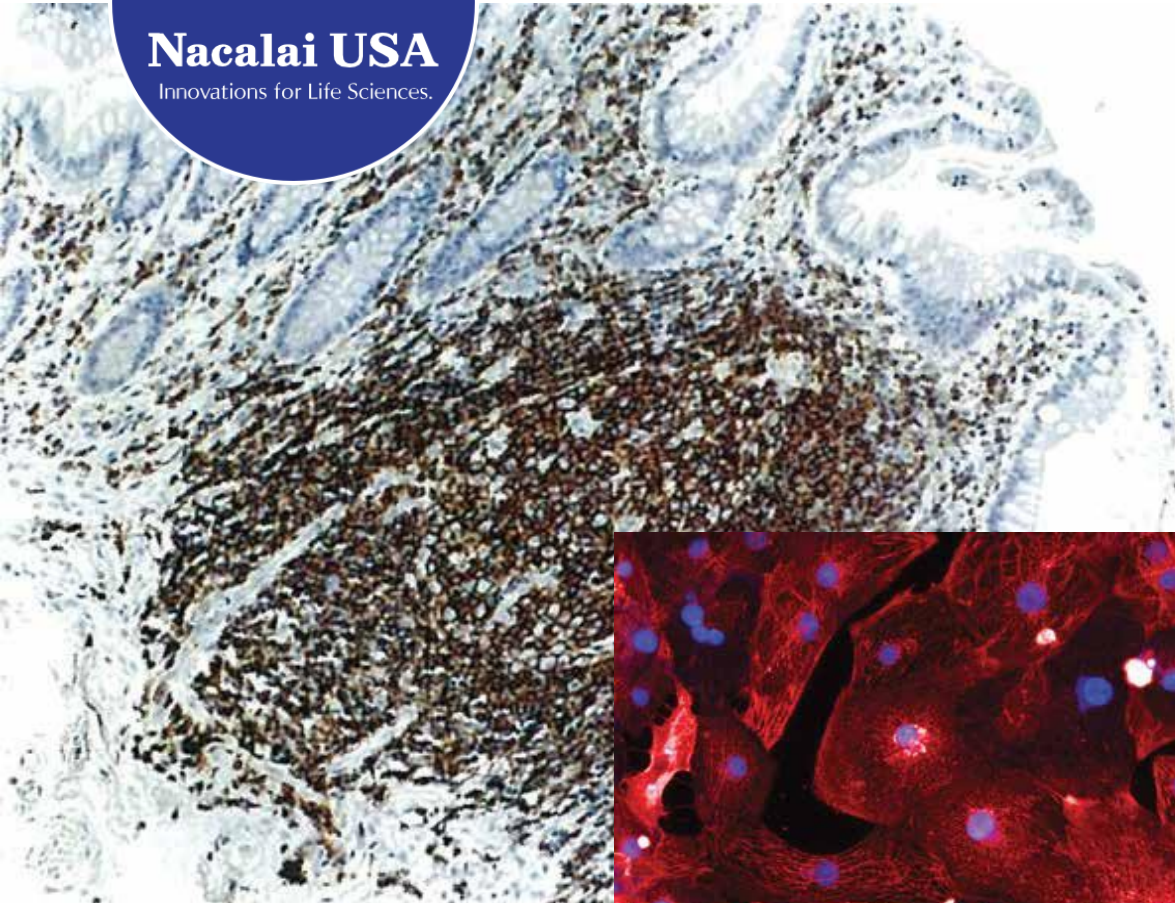


Immunohistochemistry

Immunocytochemistry



Antigen Retrieval: No damage to antigen
Blocking: High blocking efficiency
Antifade: Non-hardening
Antibody Diluent: Signal enhancer
Chromogenic Detection: Simple staining
Anti GFP antibody: Rat monoclonal antibody

Antigen Retrieval Solution

HistoVT One



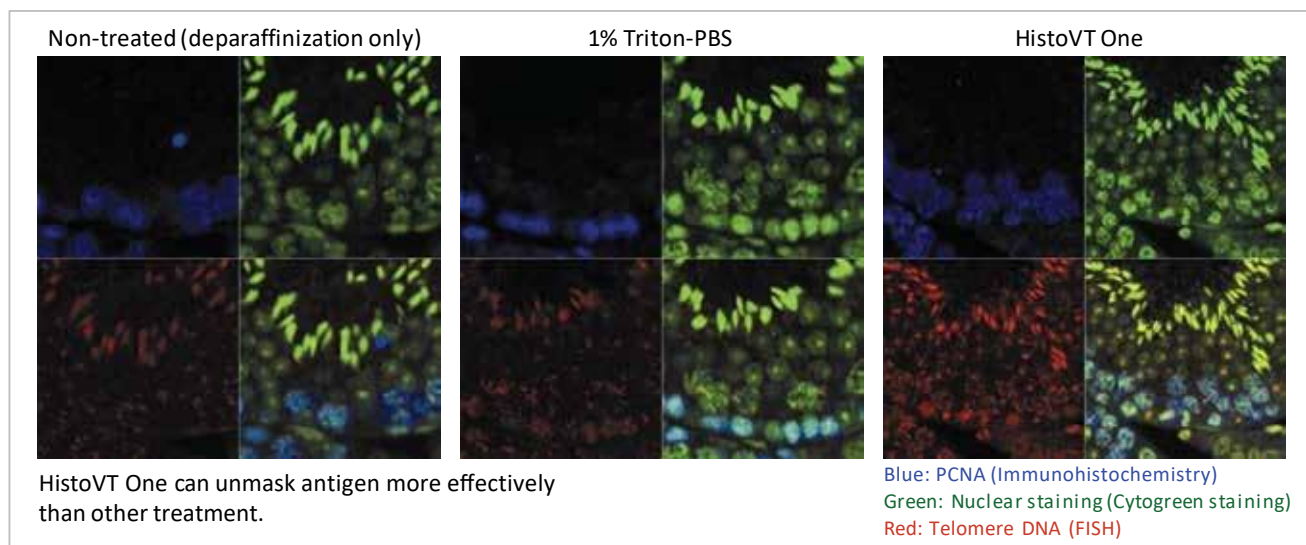
■ Features

Many references

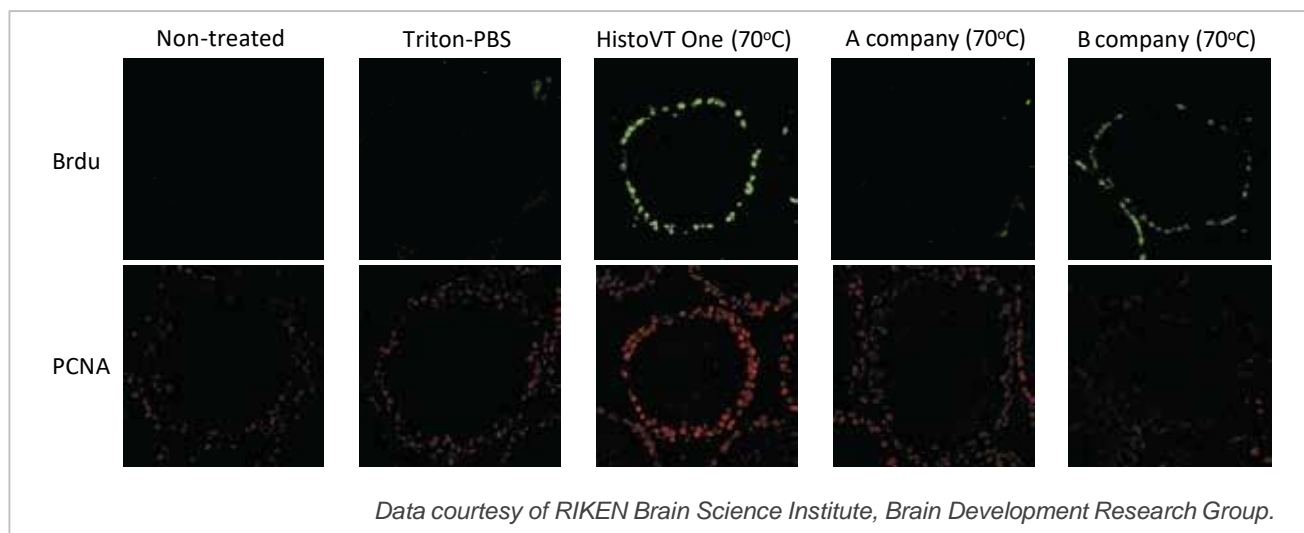
Enhancing antigen-antibody reaction

Applicable to frozen or paraffin-embedded tissue sections

■ Application data 1: Formalin-fixed, paraffin-embedded tissue sections



■ Application data 2: Frozen sections



■ Ordering Information

Product Name	Storage	Cat. No.	Quantity
HistoVT One (10X, pH 7.0)	RT	06380-05	500 ml

Blocking Solution

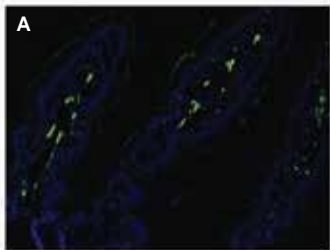
Blocking One Histo



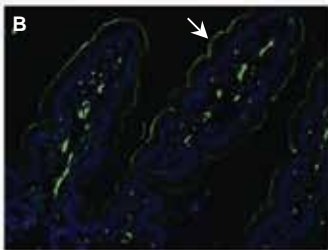
■ Features

- Easy-to-use: eye-drop bottle
- Safe: the preservative does not affect the activity of alkaline phosphatase or horseradish peroxidase

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



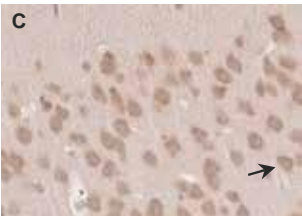
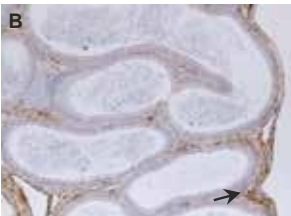
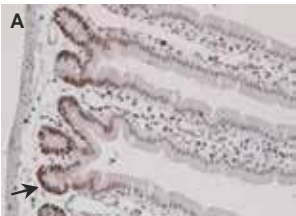
Blocking One Histo (10 min.)



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.

■ Application data



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

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 Benzydine) 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.

■ Ordering Information

Product Name	Storage	Cat. No.	Quantity
Blocking One Histo	4°C	06349-64	50 ml

Mounting Medium for Fluorescent Staining Fluoro-KEEPER Antifade Reagent, Non-hardening Type



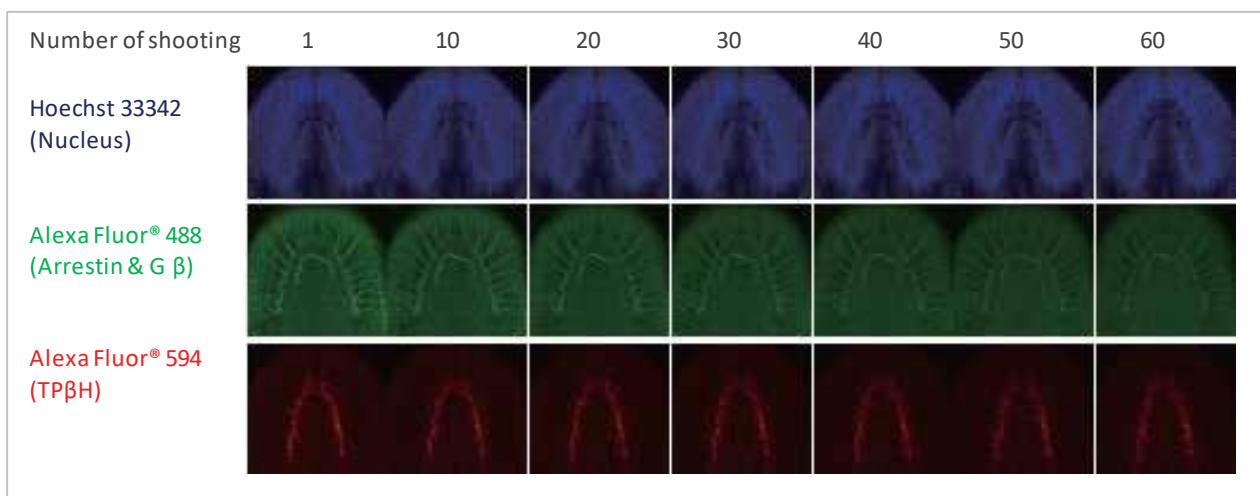
■ Features

Inhibits photobleaching of various fluorochromes
Easy to use with eye-drop bottle
Non-hardening type
Available with or without DAPI



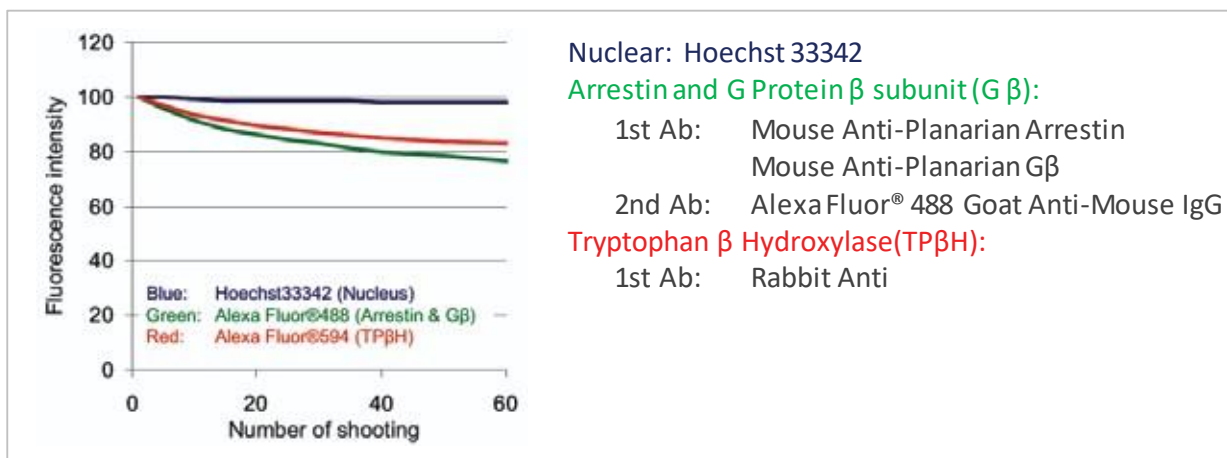
■ Fluoro-KEEPER (without DAPI)

Fluorescent microscopy experiments : Planarian



Fluorescence intensity

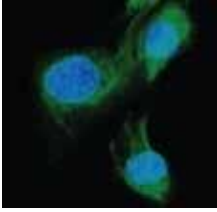
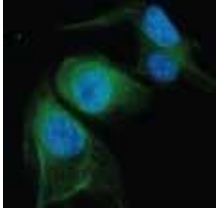
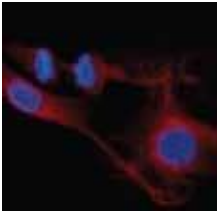
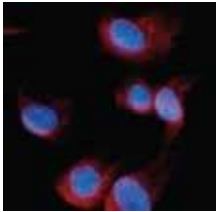
Fluorescence intensities are shown as percentages of initial intensities remaining during repeated frame capture up to 60 times. The images were acquired by Olympus FV10. The samples mounted in the Fluoro-KEEPER Antifade Reagent were clearly detected after 60 times of frame capture.



Data courtesy of Agata Lab, Department of Biophysics, Kyoto University

Fluorescent microscopy observation in 3 months

The samples mounted in Fluoro-KEEPER Antifade Reagent without DAPI can be stored for long-term when the coverslip are completely sealed with nail polish.

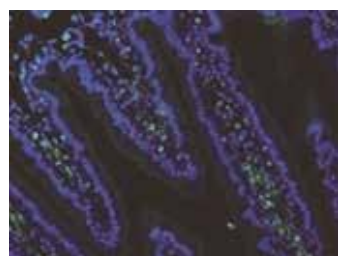
	Right after mounting	3 months later
DAPI (Nucleus) CF™ 488 (Vimentin)		
Hoechst 33342 (Nucleus) CF™ 555 (Vimentin)		

Sealing: Nail polish
Storage: 3 months at 4°C, protected from light
Sample: MC3T3-E1 cell (Fixation: 4%-Paraformaldehyde Phosphate Buffer Solution (Cat. No.: 09154) for 15 min. at RT)
Retrieval: HistoVT One (Cat. No.: 06380) for 30 min. at 90°C
Blocking: Blocking One Histo (Cat. No.: 06349)
1st Ab: Anti-Vimentin Rabbit Polyclonal Antibody (Santa Cruz, Cat. No.: sc-7557R)
2nd Ab: CF™ 488A Goat Anti-Rabbit IgG (H+L), F(ab')2 Fragment (Biotium, Cat. No.: 20013)
CF™ 588 Goat Anti-Rabbit IgG (H+L) (Biotium, Cat. No.: 20033)
Nuclear Staining: DAPI (Cat. No.: 11034), Hoechst 33258 (Cat. No.: 04907)

Fluoro-KEEPER (with DAPI)

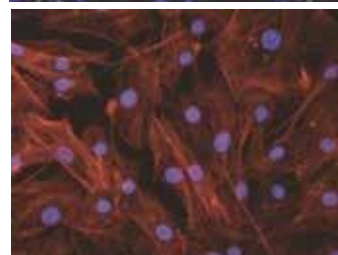
Fluorescent microscopy experiments

Mounting medium: Fluoro-KEEPER with DAPI for 30 min. at RT protecting from light
Microscopy: Olympus BX-50-34-FLA1



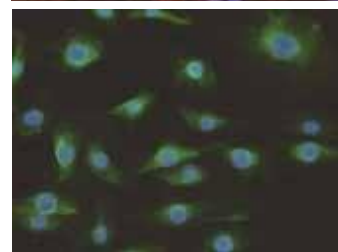
Mouse Small Intestine

Antigen Retrieval: HistoVT One (Cat. No.: 06380)
Blocking: Blocking One Histo (Cat. No.: 06349)
1st Ab: Anti-Vimentin Rabbit Polyclonal Antibody (Santa Cruz, Cat. No.: sc-7557R)
2nd Ab: CF™ 488A Goat Anti-Rabbit IgG (H+L), F(ab')2 Fragment (Biotium, Cat. No.: 20013)



MC3T3-E1 Cell

Blocking: Blocking One Histo (Cat. No.: 06349)
Rhodamine Phalloidin (Cytoskeleton, Cat. No.: PHDR1)



MC3T3-E1 Cell

Blocking: Blocking One Histo (Cat. No.: 06349)
1st Ab: Anti-Vimentin Rabbit Polyclonal Antibody (Santa Cruz, Cat. No.: sc-7557R)
2nd Ab: Cy2® Goat anti-Rabbit IgG (H+L) (Genetex, Cat. No.: GTC26940)

Antigen Retrieval

Blocking

Antifade

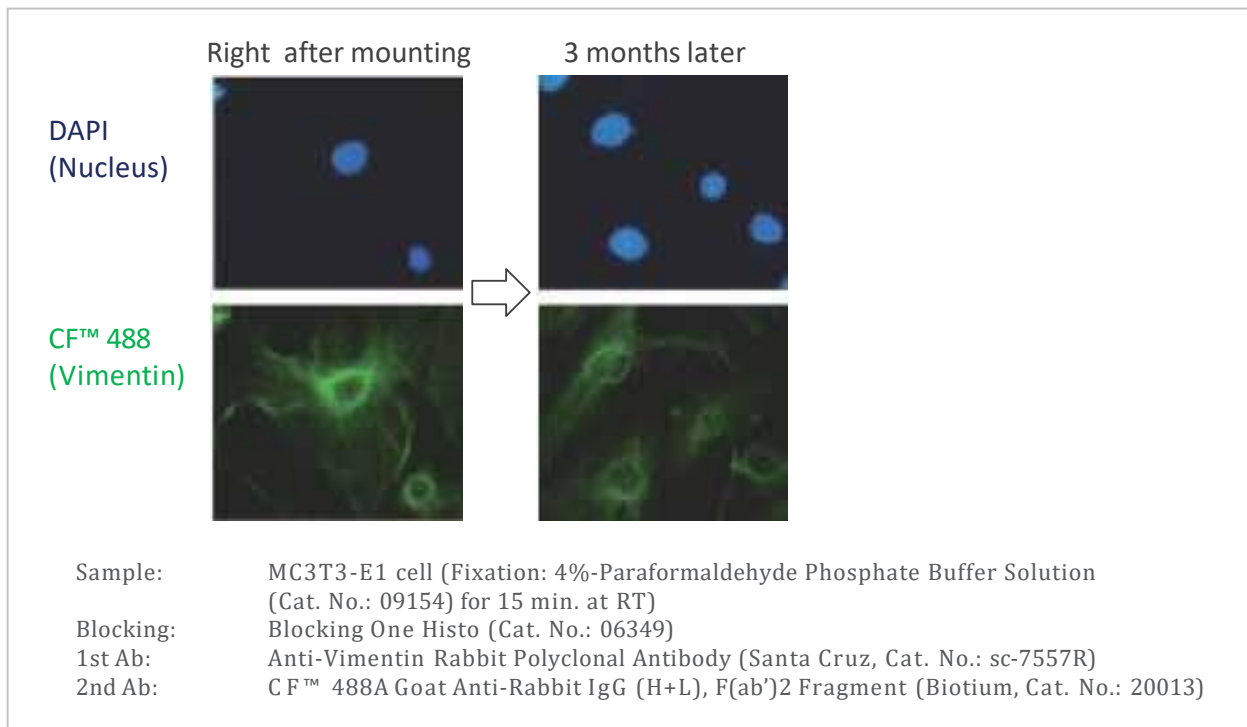
Antibody Diluent

Detection

Anti GFP Antibody

Fluorescent microscopy observation in 3 months

The samples mounted in Fluoro-KEEPER Antifade Reagent with DAPI can offer brightly signals of DAPI and CF™ 488 in spite of storage for 3 months upon sealing of a coverslip. For long-term storage, seal with hot wax or nail polish and store at 4°C protecting from light.



Comparison of antifade effectiveness with different fluorescent dyes

Cells stained by each fluorescent dye were mounted in Fluoro-KEEPER Antifade Reagent, 85% Glycerol containing PBS as a control. Samples were illuminated for 60 seconds. Each number indicates fluorescence intensity as percentage of initial intensity after 60 seconds exposure photobleaching.

Fluorescent Dye	without DAPI		with DAPI	
	Fluoro-KEEPER	Control	Fluoro-KEEPER	Control
Hoechst 33258	98 %	96 %	-	-
Hoechst 33342	100 %	90 %	-	-
DAPI	99 %	93 %	-	-
Propidium Iodide	95 %	67 %	-	-
Fluorescein	97 %	49 %	96 %	49 %
Alexa Fluor® 488	93 %	86 %	96 %	91 %
CF™ 488	93 %	82 %	91 %	82 %
Cy® 2	99 %	83 %	98 %	81 %
Rhodamine	72 %	51 %	78 %	41 %
Alexa Fluor® 555	98 %	81 %	97 %	87 %
CF™ 555	98 %	85 %	97 %	85 %
Cy® 3	89 %	71 %	86 %	66 %

Control Condition:
85 % Glycerol-PBS (without DAPI)
85 % Glycerol-PBS with DAPI

Fluorescent Microscopy:
Olympus BX-50-34-FLA1

Exposure Time:
60 seconds.

Ordering Information

Product Name	Storage	Cat. No.	Quantity
Fluoro-KEEPER Antifade Reagent, Non-Hardening Type	4°C	12593-64	2 x 5 ml
Fluoro-KEEPER Antifade Reagent, Non-Hardening Type with DAPI	4°C	12745-74	2 x 5 ml

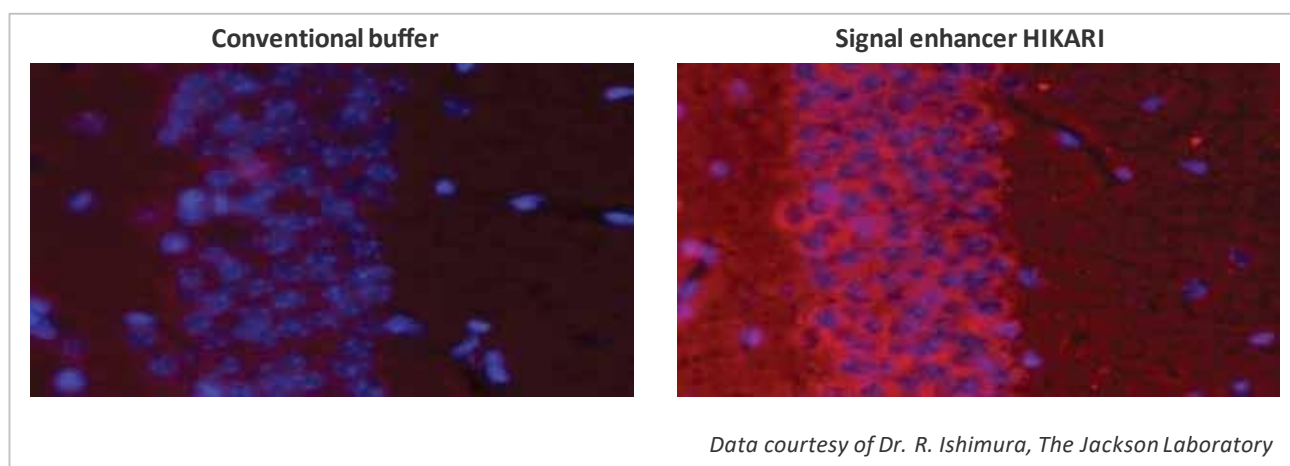


■ Features

Enhances signals - yields several fold increase in signal intensity
Reduces background - improves the specificity of your antibodies
Simple - just dilutes your antibodies with Signal Enhancer HIKARI solution
Works with any system - applicable to colorimetric, chemiluminescent and fluorescent detection

■ Application data

Brain tissue section was stained with secondary antibody conjugated with AF555 (red) and counter stained with Hoechst dye (blue).



Note: Signal Enhancer HIKARI for Immunostain Solution A and B exhibit different enhancing effects, depending on antigens and antibodies. These solutions can be used independently. However, both solutions should be explored in order to select the more suitable one for your particular assay system. A Starter Kit [NU00201] composed of both Solution A and B is available for such purpose.

■ Ordering Information

Product Name	Storage	Cat. No.	Quantity
Signal Enhancer HIKARI for Immunostain Starter Kit (Solution A & Solution B)	4°C	NU00201	5 ml each
Signal Enhancer HIKARI for Immunostain Solution A	4°C	NU00202	20 ml
Signal Enhancer HIKARI for Immunostain Solution B	4°C	NU00203	20 ml

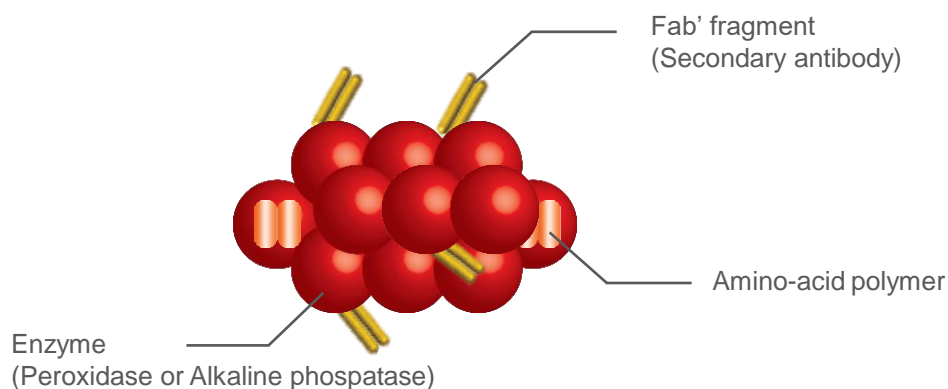
One-step Polymer Detection System

Histofine Simple Stain

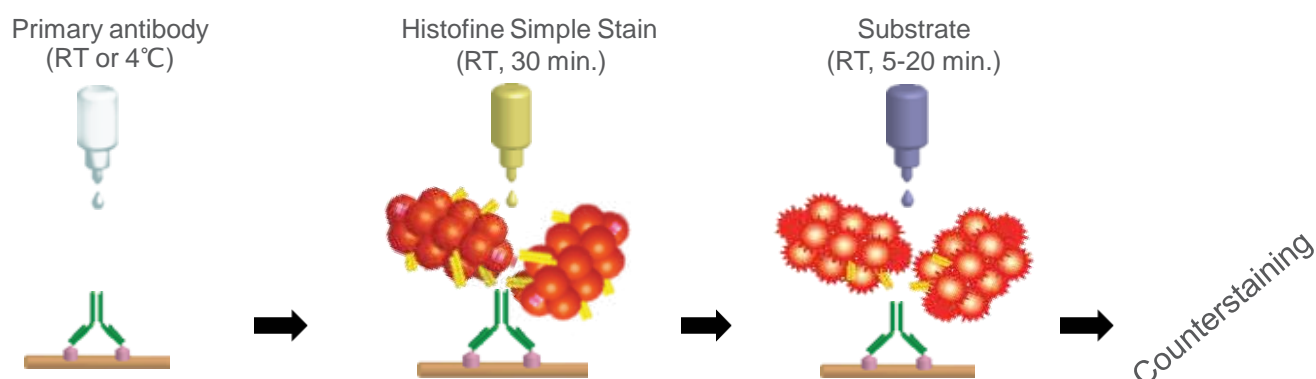


■ Polymer Technology

Multiple molecules of enzyme and secondary antibodies conjugate to amino-acid polymers. Polymeric enzyme provides increased staining intensity.

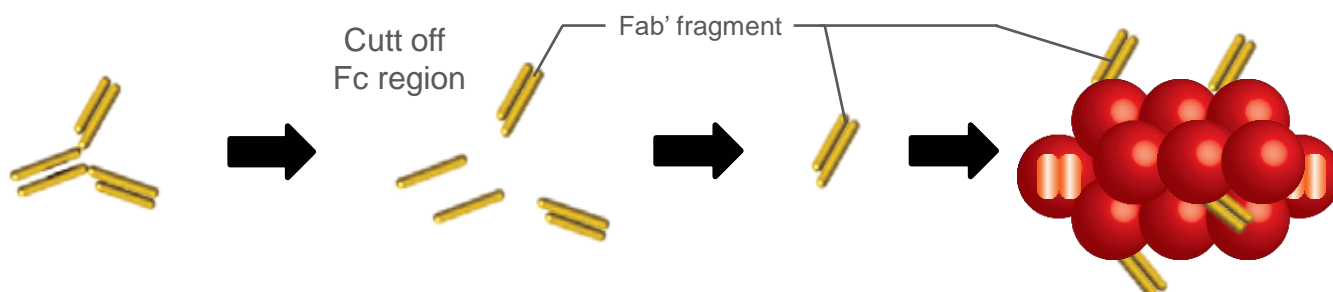


■ Simplified Staining Steps

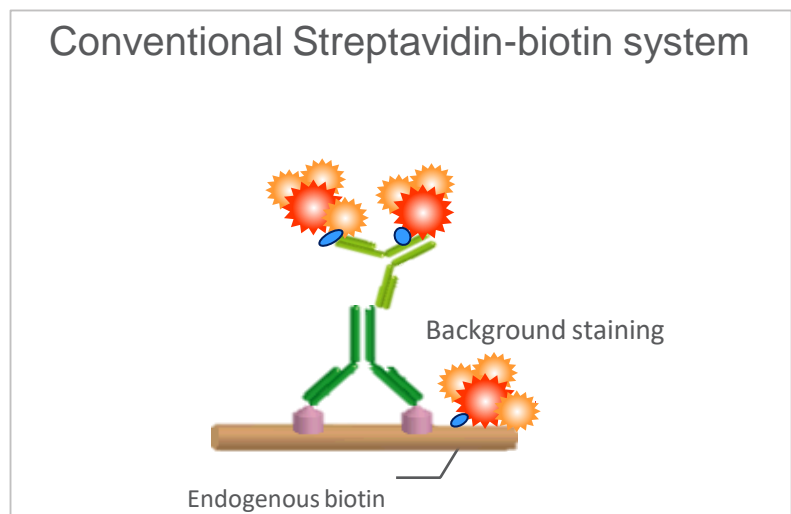
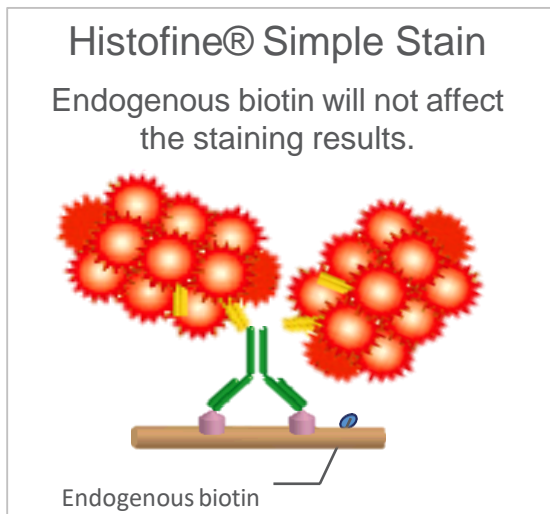


■ Low background

Only Fab' fragments are used as secondary antibodies. As Fab' fragment doesn't bind to endogenous Fc receptor, the background remains clean.

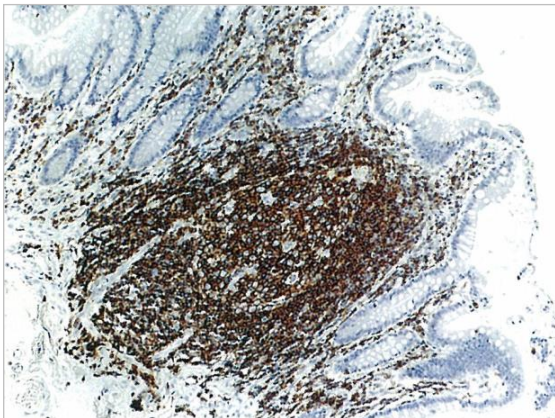


Low background



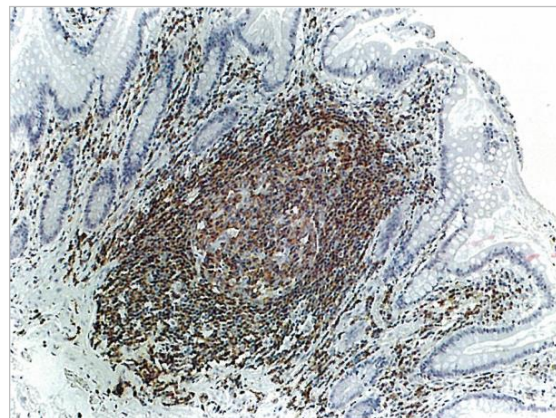
Comparison between Histofine® and conventional SAB System

Simple Stain MAX PO (M)



Primary Antibody: LCA mouse-mono
Substrate: DAB

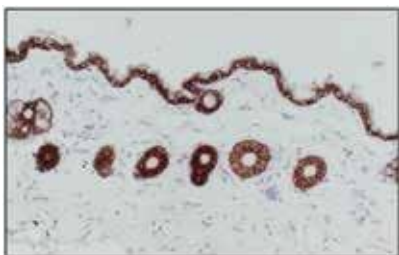
Conventional SAB System PO (M)



Primary Antibody: LCA mouse-mono
Substrate: DAB

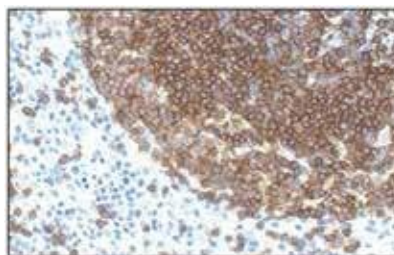
Application Data

Rabbit anti-Keratin/
Cytokeratin antibody



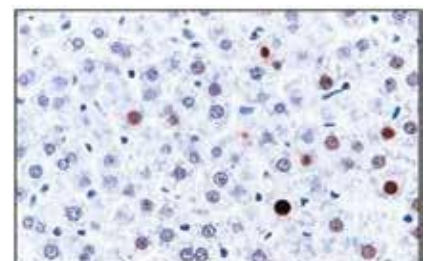
Mouse skin stained with Histofine® Simple Stain Mouse MAX PO(R) and DAB chromogen.
Note: cytoplasmic staining of epithelium cells and sweat gland cells.

Rat anti-Mouse CD45R/B220



Mouse spleen stained with Histofine® Simple Stain Mouse MAX PO(Rat) and DAB Chromogen.
Note: membrane staining of almost all lymphocytes in germinal center and scattered interfollicular lymphocytes.

Mouse anti-PCNA antibody
(clone:PC10)



Rat liver stained with Histofine® Simple Stain Rat MAX PO(MULTI) and DAB chromogen.
Note: nuclear staining of liver cells.

Ordering Information

For **Mouse** Tissue Sections

Product Name	Catalog No.	Quantity	Size	Species of primary antibody
Histofine® Simple Stain MOUSESTAIN KIT	414321F 414322F	50 tests 500 tests	6 mL ea 51 mL ea	Mouse primary antibody
Histofine® Simple Stain Mouse MAX PO (R)	414341F	170 tests	1 x 17 ml	Rat primary antibody
Histofine® Simple Stain Mouse MAX PO (G)	414351F	170 tests	1 x 17 ml	Goat primary antibody
Histofine® Simple Stain Mouse MAX PO (Rat)	414311F	170 tests	1 x 17 ml	Rat primary antibody

For **Rat** Tissue Sections

Histofine® Simple Stain Rat MAX PO (MULTI)	414191F	170 tests	1 x 17 ml	Mouse & rabbit primary ab
Histofine® Simple Stain Rat MAX PO (M)	414171F	170 tests	1 x 17 ml	Mouse primary antibody
Histofine® Simple Stain Rat MAX PO (R)	414181F	170 tests	1 x 17 ml	Rabbit primary antibody
Histofine® Simple Stain Rat MAX PO (G)	414331F	170 tests	1 x 17 ml	Goat primary antibody

For **Human** Tissue Sections

Histofine® High Stain Human HRP (MULTI) -High Sensitive 2 Step Polymer Detection System	414481F 414483F	170 tests 1000 tests	1 x 17 ml ea 6 x 17 ml ea	Mouse & rabbit primary ab
Histofine® Simple Stain Human MAX PO (MULTI)	414151F 414152F 414154F	170 tests 500 tests 1500 tests	1 x 17 ml 3 x 17 ml 9 x 17 ml	Mouse & rabbit primary ab
Histofine® Simple Stain Human MAX PO (M)	414131F 414132F 414134F	170 tests 500 tests 1500 tests	1 x 17 ml 3 x 17 ml 9 x 17 ml	Mouse primary antibody
Histofine® Simple Stain Human MAX PO (R)	414141F 414142F 414144F	170 tests 500 tests 1500 tests	1 x 17 ml 3 x 17 ml 9 x 17 ml	Rabbit primary antibody
Histofine® Simple Stain Human MAX PO (G)	414161F 414162F	170 tests 500 tests	1 x 17 ml 3 x 17 ml	Goat primary antibody
Histofine® Simple Stain Human AP (MULTI)	414261F 414262F	170 tests 500 tests	1 x 17 ml 3 x 17 ml	Mouse & rabbit primary ab
Histofine® Simple Stain Human AP (M)	414241F 414242F	170 tests 500 tests	1 x 17 ml 3 x 17 ml	Mouse primary antibody
Histofine® Simple Stain Human AP (R)	414251F 414252F	170 tests 500 tests	1 x 17 ml 3 x 17 ml	Rabbit primary antibody

Detection Kit

Histofine® DAB-3S Kit	415192F 415194F	500 tests 1500 tests	1 x 3 ml 3 x 9 ml	Peroxidase
Histofine® DAB-2V Kit	425312F 425314F	500 tests 1500 tests	- -	Peroxidase
Histofine® Simple Stain AEC Solution	415182F 415184F	500 tests 1500 tests	- -	Peroxidase
Histofine® New Fuchsin Substrate Kit	415161F	2000 tests	-	Alkaline phosphatase
Histofine® ALK Detection Kit	417071F	20 tests	-	
Histofine® ALK Control Slides	417081F	5 slides	-	

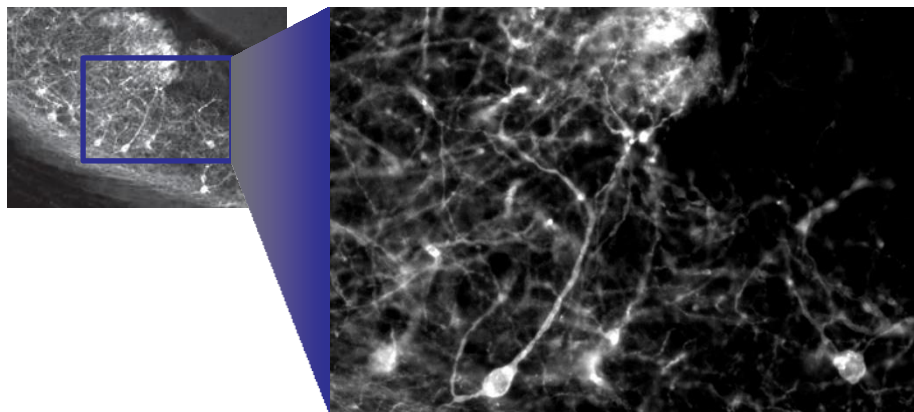
Histofine® primary antibodies

Histofine® primary antibodies for human tissue sections are applicable to FFPE tissue sections and used for histopathological differentiation. Pretreatment method and dilution rate are indicated on our web site.

Rat anti-GFP Antibody, Mono



Application



Data courtesy of Dr. Y. Yoshihara, RIKEN Brain Science Institute

Sample : Mouse brain (nerve cell)
Primary antibody : Anti-GFP (Rat IgG2a), Mono (1:1000) RT, over night
Secondary antibody : Anti-Rat IgG-Cy3 (1:300)
Blocking : RT, 1 hr
Fixing method : 5% Normal goat serum/
0.2% TritonX-100 in PBS
4% Paraformaldehyde

Product Specification

Clone : GF090R
Isotype : IgG2a (Rat)
Product form : Liquid
Immunogen : His-GFP (full length) fusion protein
Application : Immunohistochemistry 1:1000-1:2000
Western Blotting 1:1000-1:2000
ELISA 1:2000-1:20000

Reference

Yonehara, K. Nature. 469(7330):407-10 (2011)
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Vidaki, M. et al. Cereb Cortex. (2011)
Konno, A. et al. Neurosci Res. (2011)
Gotoh, H. et al. Dev Biol. 349(2):504-11 (2011)

Ordering Information

Product Name	Storage	Cat. No.	Qty.
Anti-GFP (Rat IgG2a), Monoclonal (GF090R)	4°C	04404-84	200 µg

Antigen Retrieval

Blocking

Antifade

Antibody Diluent

Detection

Anti-GFP Antibody

NACALAI USA, INC.

6625 Top Gun Street, Suite 107
San Diego, CA 92121



TEL; 858.404.0403

E-mail; info@nacalaiusa.com

Web; www.nacalaiusa.com

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