



Mounting Medium for Fluorescent Staining

Fluoro-KEEPER Antifade Reagent, Non-Hardening Type

Fluoro-KEEPER Antifade Reagent is a non-hardening type mounting medium with a unique antifade reagent. It suppresses rapid photobleaching during fluorescence microscopy experiments. The coverslipped slide with nail polish or other sealants can be stable for several weeks. There are two types of products available, with DAPI [4',6-Diamidino-2-phenylindole] and without DAPI, which counterstains nucleus blue.

Features

- ▶ Inhibits photobleaching of various fluorescent dyes
- ▶ Easy to use with eye-drop bottle
- ▶ Available with or without DAPI



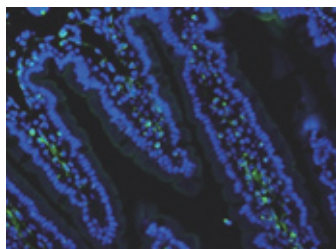
Fluoro-KEEPER (with DAPI)

Fluorescent microscopy experiments

Fluoro-KEEPER with DAPI offers nuclear staining along with mounting.

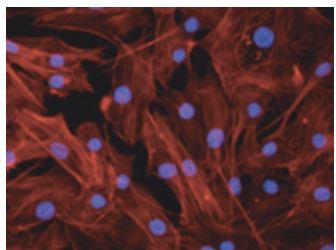
Mounting Medium: Fluoro-KEEPER with DAPI for 30 min. at room temperature protecting from light.
Microscopy: Olympus BX-50-34-FLA1

Mouse Small Intestine



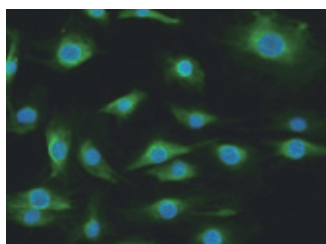
Antigen retrieval : HistoVT One (#06380)
Blocking : Blocking One Histo (#06349)
1st Ab : Anti-Vimentin Rabbit Polyclonal Antibody (Santa Cruz #sc-7557R)
2nd Ab : CF™ 488A Goat Anti-Rabbit IgG (H+L), F(ab')₂ Fragment (Biotium #20013)

MC3T3-E1 Cell



Blocking : Blocking One Histo (#06349)
Rhodamine-conjugated phalloidin (Cytoskeleton #PHDR1)

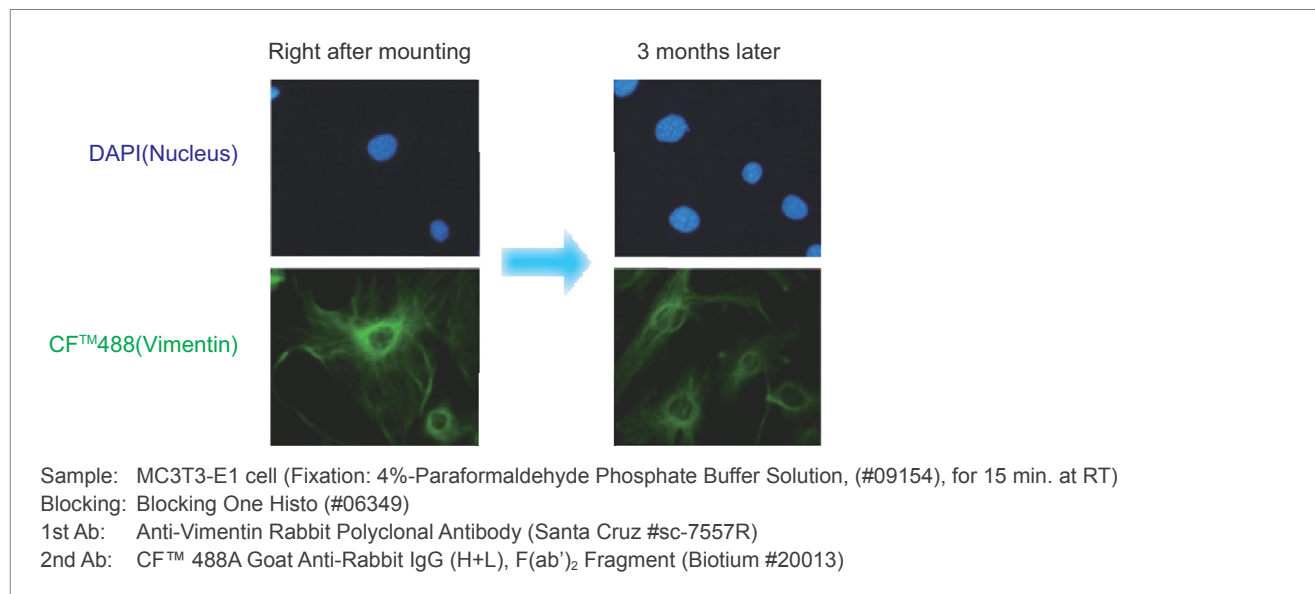
MC3T3-E1 Cell



Blocking : Blocking One Histo (#06349)
1st Ab : Anti-Vimentin Rabbit Polyclonal Antibody (Santa Cruz #sc-7557R)
2nd Ab : Cy[®]2 Goat Anti-Rabbit IgG (H+L) (GENETEX #GTX26940)

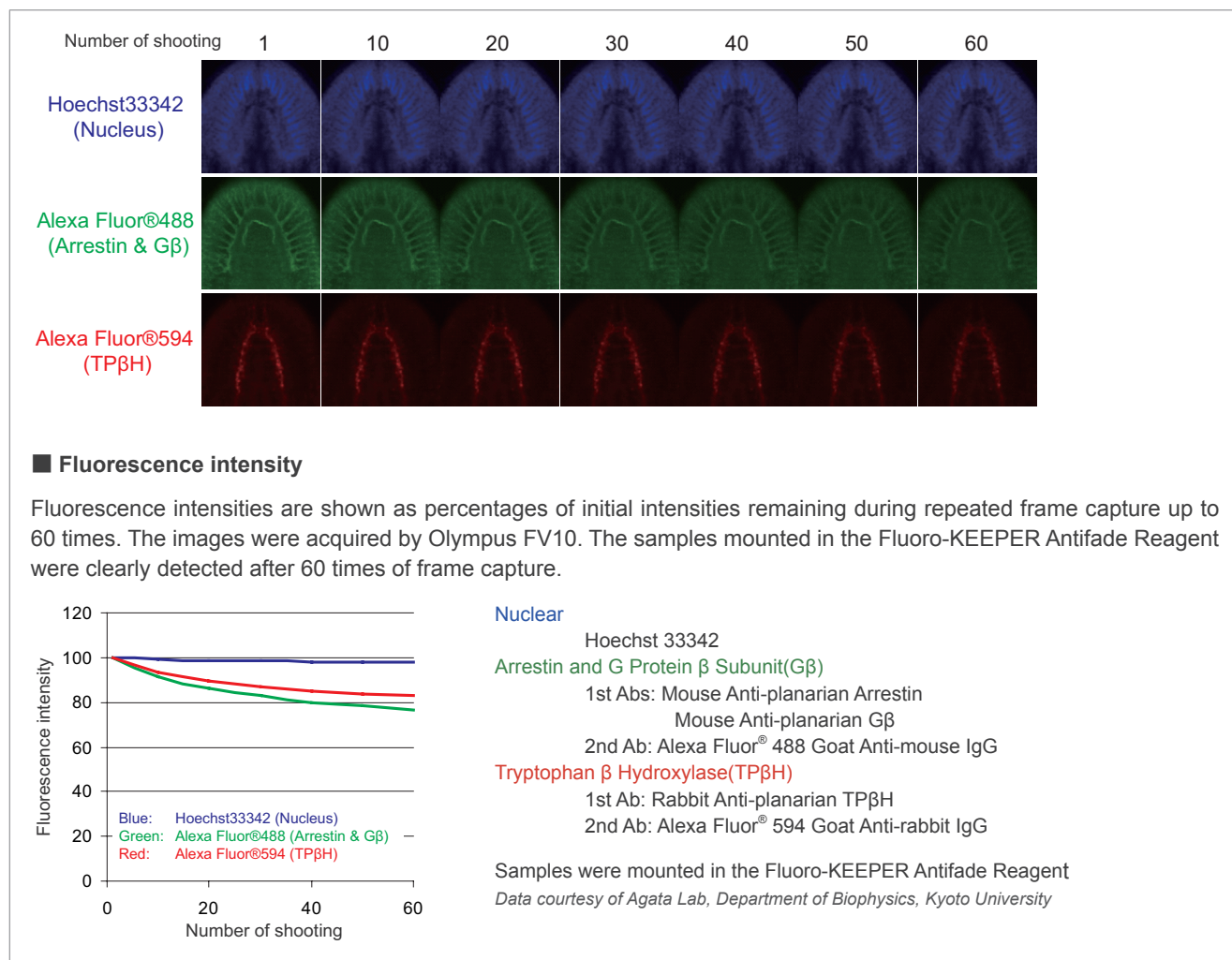
Fluorescent microscopy observation in three months

The samples mounted in Fluoro-KEEPER Antifade Reagent with DAPI can offer brightly signals of DAPI and CF488™ in spite of storage for three 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. Please refer to the instruction for more details.



Fluoro-KEEPER (without DAPI)

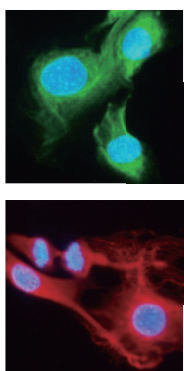
Fluorescent microscopy experiments; Planarian



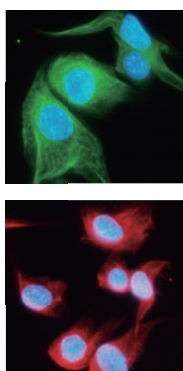
Fluorescent microscopy observation in three months

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



Sealing: Nail polish

Storage: 3 months at 4 °C protected from light

Sample: MC3T3-E1 cell (Fixation: 4%-Paraformaldehyde Phosphate Buffer Solution, (#09154), for 15 min. at RT)

Retrieval: Histo VT One (#06380) for 30 min. at 90°C

Blocking: Blocking One Histo (#06349)

1st Ab: Anti-vimentin Rabbit Polyclonal Antibody (Santa Cruz #sc-7557R)

2nd Ab: CF™ 488A Goat Anti-rabbit IgG(H+L), F(ab')2 Fragment (Biotium #20013) CF™ 555 Goat Anti-rabbit IgG (H+L(Biotium #20033))

Nuclear Staining: DAPI (#11034) or Hoechst33258 (#04907)

Comparison of antifade effectiveness with different fluorescent dyes

Fluoro-KEEPER Antifade Reagent offers increased resistance to photobleaching of various fluorescent dyes.

	without DAPI		with DAPI	
Fluorescence Dye	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

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.

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

Fluorescent Microscopy: Olympus BX-50-34-FLA1

Exposure time: 60 seconds.

Protocol

1. Remove excess liquid from a slide and dispense a couple of drops of the product onto a specimen.
2. Cover the specimen with a coverslip.
3. The slide can be observed immediately.
4. To obtain sufficient counterstain, incubate the slide for 30 min. at room temperature protecting from light.

For long-term storage, sealing with hot wax or nail polish and store at 4 °C protected from light.

Precaution

1. Because the product contains glycerol, it is not recommended for the detection of phycobiliproteins such as phycoerythrin and allophycocyanin.
2. Refractive index of Fluoro-KEEPER Antifade Reagent with DAPI and without DAPI is 1.43 - 1.44(20°C)

Ordering Information

Product Name	Grade	Storage	Product No.	PKG Size
Fluoro-KEEPER Antifade Reagent, Non-Hardening Type	SP	R	12593-64	2 x 5 ml
Fluoro-KEEPER Antifade Reagent, Non-Hardening Type with DAPI	SP	R	12745-74	2 x 5 ml

Related Products

Product Name	Grade	Storage	Product No.	PKG Size
Fixation				
10% - Formaldehyde Neutral Buffer Solution	-	RT	37152-51	1 L
4% - Paraformaldehyde Phosphate Buffer Solution	SP	R	09154-14	5 x 10 ml
			09154-85	500 ml
Blocking Reagents				
Blocking One Histo	SP	R	06349-64	50 ml
Antigen Retrieval				
HistoVT One(10x, pH 7.0)	SP	RT	06380-05	500 ml

(Storage) RT: Room temperature R: Refrigerator

Antigen retrieval solution

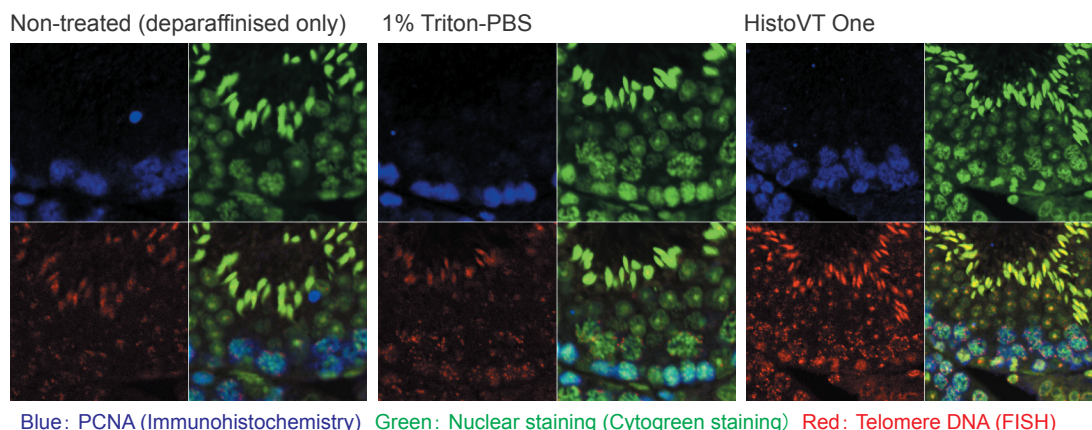
HistoVT One

HistoVT One is an antigen retrieval solution for immunohistochemistry and in situ hybridization. This product can unmask antigenic sites without damage to antigens in formalin-fixed, frozen or paraffin-embedded tissue sections.

- *Enhancing antigen-antibody reaction*
- *Applicable to frozen or paraffin-embedded tissue sections*
- *High reproducibility*

Application

Formalin-fixed, paraffine-embedded tissue sections.



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

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