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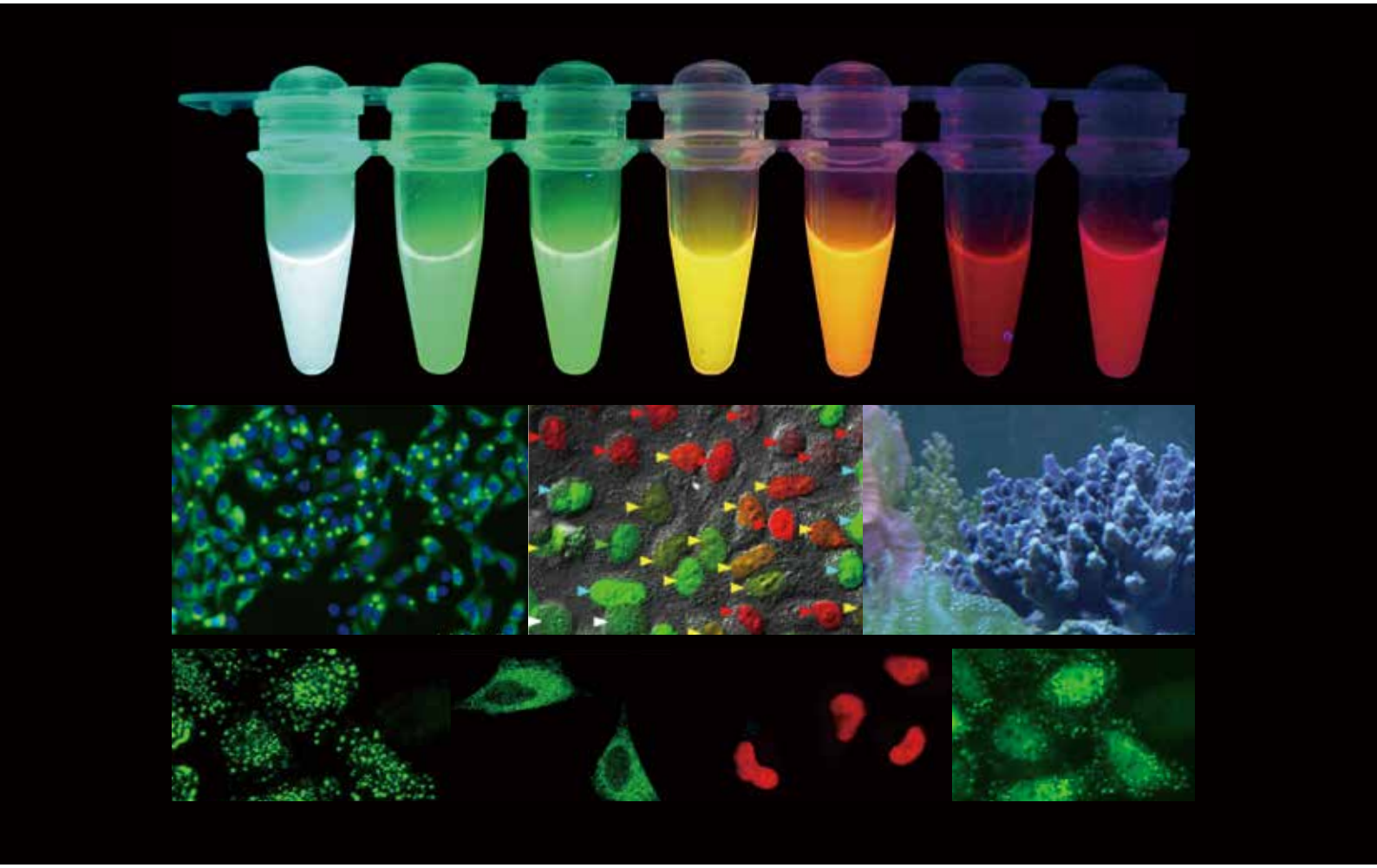
MBL
A JSR Life Sciences Company

Amalgaam
Fluorescent proteins



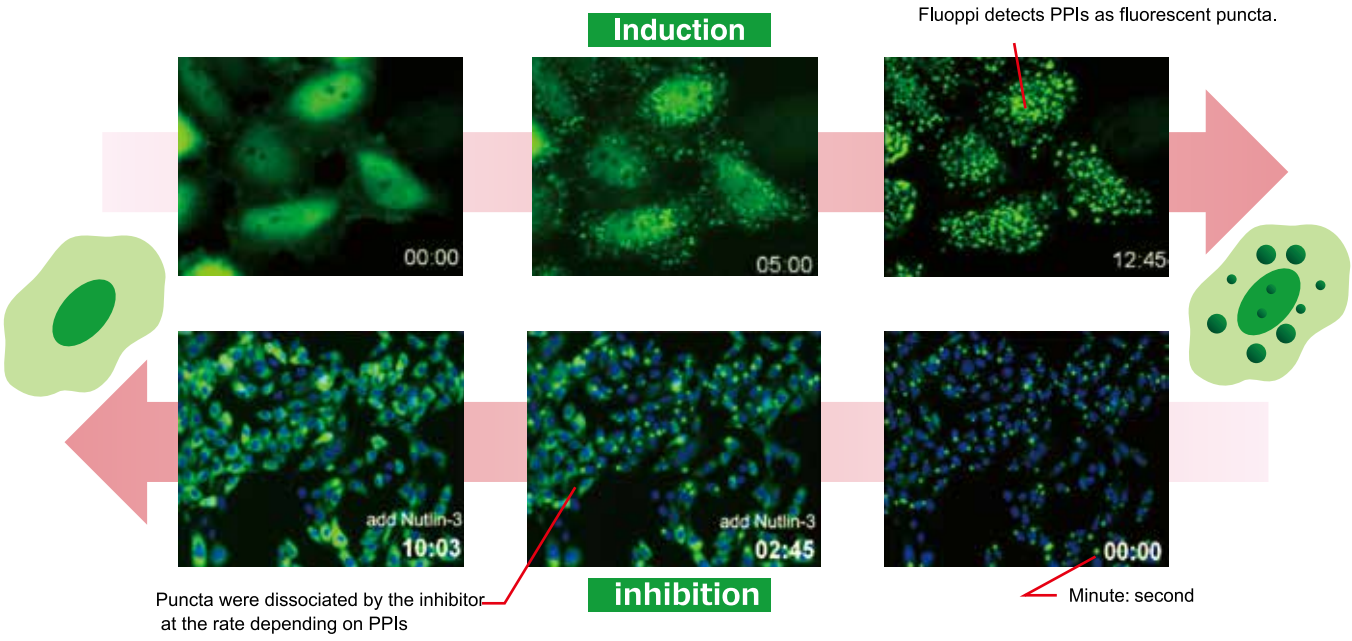
- Protein-Protein Interactions Detection System
- Advanced Fluorescent Indicator
- Basic Fluorescent Proteins
- Antibodies

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

Fluoppi

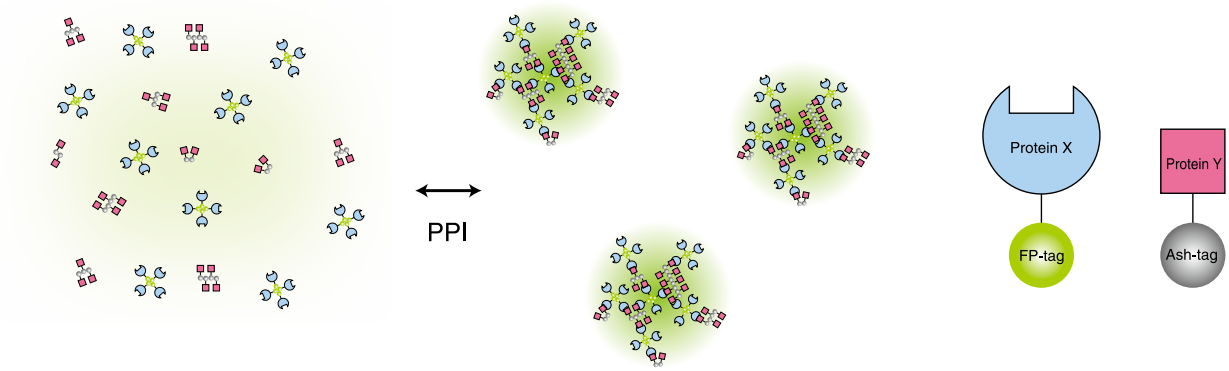
Novel technology for detecting PPIs

- **Visualize PPI as fluorescent "Puncta" in living cells**
- **"Reversible" puncta-formation**
- **Easy to construct an experimental system**
- **Ideal tool for drug screening**



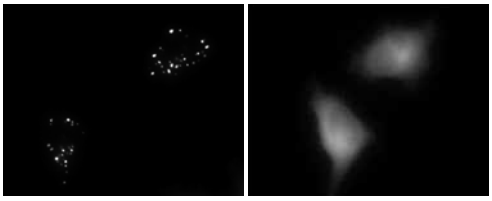
Mechanism of action

Fluoppi is a technology providing an easy way to visualize protein-protein interactions (PPIs) with a high signal to noise ratio. It employs an oligomeric assembly helper tag (Ash-tag) and a tetrameric fluorescent protein tag (FP-tag) to create detectable fluorescent puncta when there are interactions between two proteins fused to the tags. By way of example, genetic fusion of protein X with FP-tag, and Y with Ash-Tag creates a tetrameric fluorescent fusion protein X-FP and an oligomeric fusion protein Y-Ash respectively. Because each fusion protein has multiple Xs or Ys, interaction between protein X and Y causes large lattice like complexes where the fluorescence by X-FP is concentrated and detectable as fluorescent puncta.

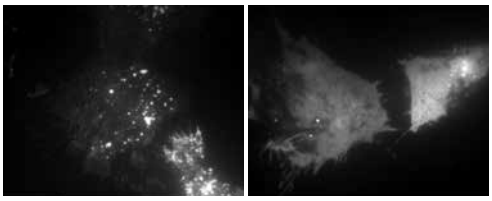


Localization

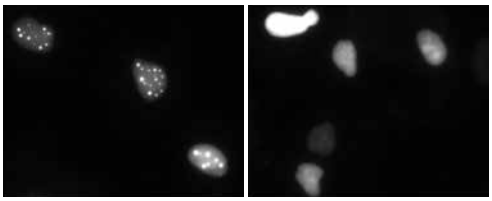
Because location of puncta is not restricted to specific site inside the cell, Fluoppi can detect PPIs at several subcellular localizations such as cytosol, nucleus, and juxtamembrane.
The left pictures represent puncta at several subcellular localizations, and the right pictures are negative controls which express hAG tagged protein and Ash-tag without fusing any proteins.
The images of juxtamembrane are taken by Total Internal Reflection Fluorescence Microscopy (TIRFM).



PPI in the cytosol　　Negative control



PPI at juxtamembrane　　Negative control

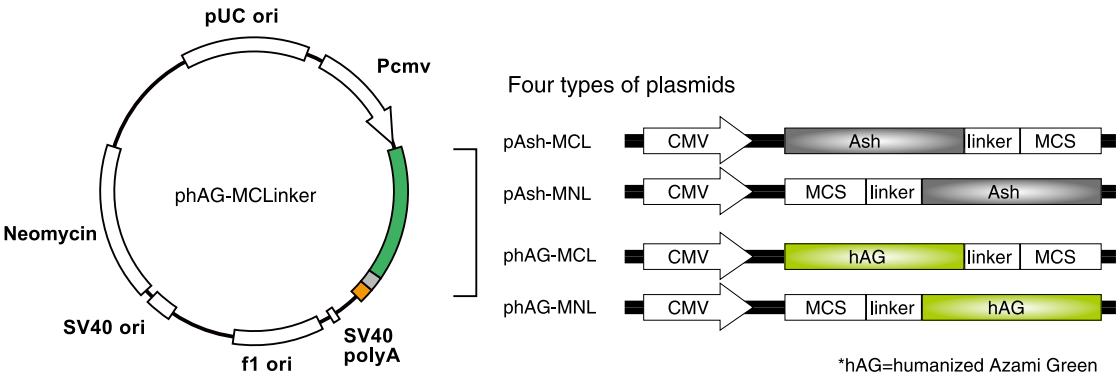


PPI in the nucleus　　Negative control

Workflow

Fluoppi tags are able to work in both *N* and *C* terminal fusion. We have several plasmid vectors which include CMV promoter, Fluoppi tag, flexible peptide linker, Multiple Cloning Site (MCS) and Neomycin resistant gene.
At first, proteins X & Y of your interest are fused to FP-tag and Ash-tag respectively. We recommend to prepare all the eight possible constructs to identify the best workable combination.

Because fluorescent signal of Fluoppi is very high, conventional fluorescence microscopy can be used to image the cell.
If the proteins interact with each other upon expression, fluorescent puncta will be detected. Formation of puncta is reversible so that they can be dissociated and the fluorescent signal will spread over the cell by PPI inhibitors, and vice versa by PPI inducers.



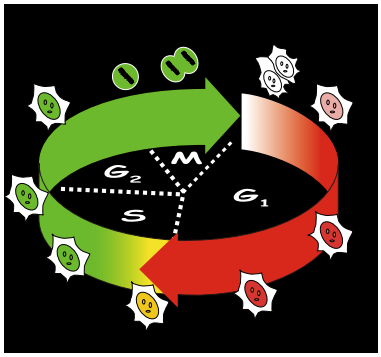
"Flexible"peptide linker (22 aa):
N term.- NSADG GGGSG GSGGS GGGST QG – *C* term.

Fluorescent proteins	Code No.	Products	Volume
Monti-Red (Red)	AM-8012M	Fluoppi Ver.2 : Ash-Red (Ash-MNL/MCL + Monti-Red-MNL/MCL)	10 μg each
	AM-VS0802M	Monti-Red for Fluoppi (pMonti-Red-MNL/MCL)	10 μg each
hAG (Green)	AM-8011M	Fluoppi Ver.2 : Ash-hAG (Ash-MNL/MCL + hAG-MNL/MCL)	10 μg each
	AM-8201M	Fluoppi : Ash-hAG [p53-MDM2]	10 μg each
	AM-8202M	Fluoppi : Ash-hAG [mTOR-FKBP12]	10 μg each
	AM-VS0801M	humanized Azami-Green for Fluoppi (phAG-MNL/MCL)	10 μg each

- This kit consists of four types of plasmids (pAsh-MCL, pAsh-MNL, phAG-MCL, phAG-MNL)
- The use of these products requires a license from MBL Co., Ltd. MBL grants non-profit research organizations the right to use the product for non-commercial research purpose. For commercial entities a commercial license is required. For more information, please contact support@mbl.co.jp
- Fluoppi does not guarantee detection of all Protein-Protein Interactions.
- The fluorescent proteins used in product, hAzami-Green and Monti-Red, differ from each other in fluorescence and other properties.

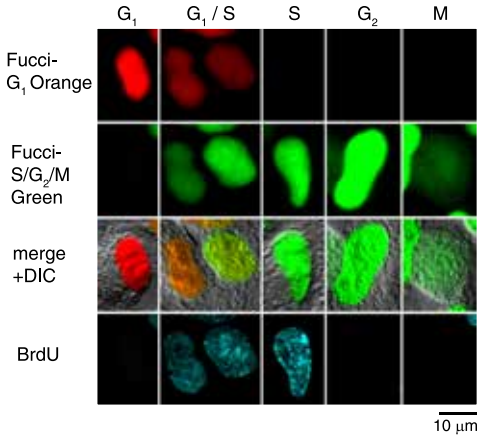
Fucci (Fluorescent Ubiquitination-based Cell Cycle Indicator)

- **Real-time visualization of cell-cycle progression**
- **Spatio-temporal imaging of cell cycle dynamics**



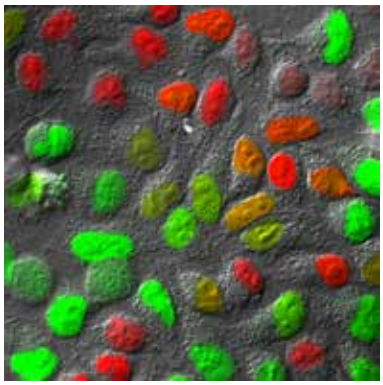
Fluorescent ubiquitination-based cell cycle indicator (Fucci) is a sophisticated technology which can visualize G_1 and/or S/G_2/ M phases of cell cycle in living cell. The mechanism of action of Fucci is based on ubiqitin-proteasome protein degradation system.
Fucci is a set of fluorescent probes: Fucci-G_1 Orange and Fucci-S/G_2/M Green. Fucci-G_1 Orange is a fusion protein of a fragment of human Cdt1 (amino acids 30-120) with the orange fluorescent mKO2 (monomeric Kusabira-Orange 2) that indicates the G_1 phase. Fucci-S/G_2/M Green is a fusion protein of a fragment of human Geminin (amino acids 1-110) with the green fluorescent protein mAG1 (monomeric Azami-Green 1) that visualizes S, G_2 and M phases.

Fluorescent images of Fucci cells



Each cell cycle of the G_1, G_1/S , S, G_2, and M phases can be determined by the combination of Fucci-G_1 Orange, Fucci-S/G_2/M Green, and an antibody against PCNA. G_1 phase is indecated by orange. Both orange and green were observed in the G_1/S phase. Additional immunostaining color by PCNA was observed at the initiation of the S phase. Cells with pure green fluorescence were either in the S or G_2 phase and were distinguished by immunostaining of the S phase. The rest of the cells were classified into the M phase.

Fucci stable transfectant (HeLa cells)



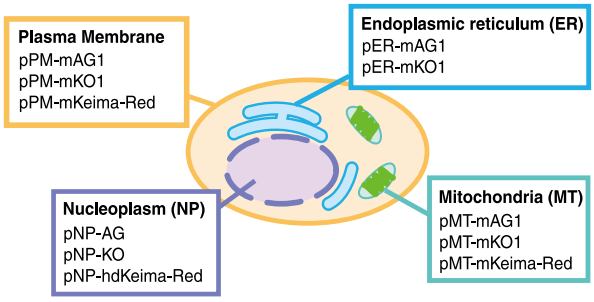
This product is licensed from RIKEN and the Tokyo Metropolitan Institute of Medical Science.

Volume: 20 μg (In cese of Vector set, each 20 μg)

Code No.	Products
AM-V9001M	pFucci-G_1 Orange (Cloning vector)
AM-V9003M	pFucci-G_1 Orange (Expression vector)
AM-V9010M	pFucci-S/G_2/M Green-Hyg (Expression vector)
AM-V9014M	pFucci-S/G_2/M Green (Cloning vector)
AM-V9016M	pFucci-S/G_2/M Green (Expression vector)
AM-V9030M	pFucci-S/G_2/M Green(N+C)-Hyg (Expression vector)
AM-V9034M	pFucci-S/G_2/M Green(N+C) (Cloning vector)
AM-VS0601M	Fucci Cloning vector Set (Orange+Green)
AM-VS0605M	Fucci Cloning vector Set (Orange+Green (N+C)
AM-VS0607M	Fucci Expression vector Set (Orange+Green-Hyg)
AM-VS0608M	Fucci Expression vector Set (Orange+Green (N+C)-Hyg)

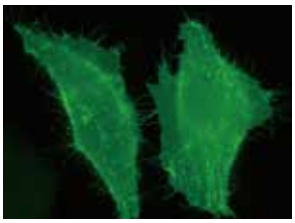
Organelle targeting vectors

- **Tools for labeling of subcellular structure**
- **Multi-labeling cells with different colors**

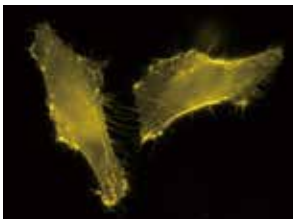


CoralHue™ organelle targeting vectors allowing to visualize subcellular structures encode fusion proteins of fluorescent proteins and localization signal sequences.
It can be used for fluorescent labelling of subcellular structures in living cells.

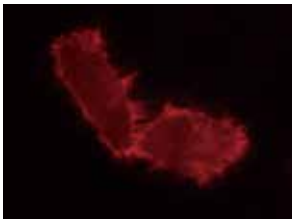
Plasma membrane (PM)



CoralHue™ pPM-mAG1

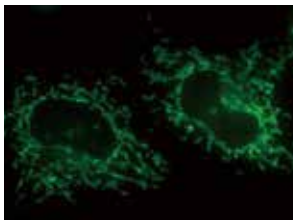


CoralHue™ pPM-mKO1

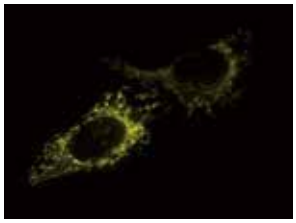


CoralHue™ pPM-mKeima-Red

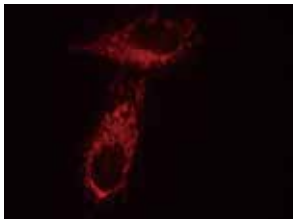
Mitochondria (MT)



CoralHue™ pMT-mAG1



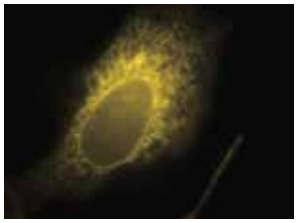
CoralHue™ pMT-mKO1



CoralHue™ pMT-mKeima-Red

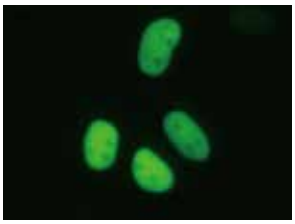
Endoplasmic reticulum (ER)

CoralHue™ pER-mAG1

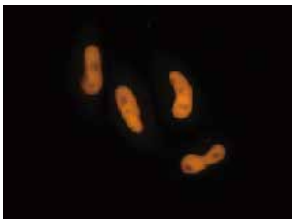


CoralHue™ pER-mKO1

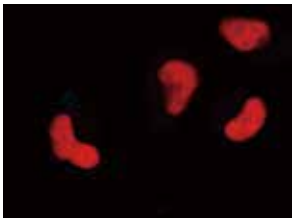
Nucleoplasm (NP)



CoralHue™ pNP-AG



CoralHue™ pNP-KO1



CoralHue™ pNP-hdKeima-Red

Volume : 20 μg

Fluorescenet protein	Abbr.	Form	Excitation maxima (nm)	Emission maxima (nm)	Mitochondria	Endoplasmic reticulum	Plasma membrane	Nucleoplasm	β-Actin
Azami -Green	AG	Tetramer	492	505				AM-V0214M	
	mAG1	Monomer	492	505	AM-V0201M	AM-V0202M	AM-V0203M		AM-V0205M
	mAG407	Monomer	407	498		AM-V0292M			
Kusabira -Orange	KO1	Dimer	548	561				AM-V0234M	
	mKO1	Monomer	548	559	AM-V0221M	AM-V0222M	AM-V0223M		AM-V0225M
Keima -Red	mKeima-Red	Monomer	440	620	AM-V0251M		AM-V0253M		
	hdKeima-Red	Dimer	440	616				AM-V0274M	
	hdKeima570	Dimer	440	570				AM-V0324M	
Midoriishi-Cyan	mMiCy1	Monomer	470	496	AM-V0261M				
Kusabira-Cyan	hKCy1	Dimer	453	486				AM-V0284M	

CoralHue™ is a product of co-development with Dr. Atsushi Miyawaki at the Laboratory for Cell Function and Dynamics, the Brain Science Institute, and the Institute of Physical and Chemical Research (RIKEN).

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CoralHue™ Fluorescent protein vectors

Volume: 20 μg

Fluorescenet protein	Abbr.	Form	Excitation maxima (nm)	Emission maxima (nm)	S1	MC1	MN1	MCLinker	MNLinker
Kusabira-Cyan	KCy1	Dimer	453	486	AM-V0171M				
Midoriishi -Cyan	MiCy1	Dimer	472	495	AM-V0061M				
	mMiCy1	Monomer	470	496	AM-V0111M				
	hmMiCy1	Monomer	470	496		AM-V0115M	AM-V0116M	AM-V0119M	AM-V0110M
Umikinoko -Green	mUKG1	Monomer	483	499	AM-V0161M				
	hmUKG1	Monomer	483	499	AM-V0164M	AM-V0165M	AM-V0166M		
Azami-Green	AG	Tetramer	492	505	AM-V0021M				
	mAG1	Monomer	492	505	AM-V0031M	AM-V0032M	AM-V0033M		
	hmAG1	Monomer	492	505	AM-V0034M	AM-V0035M	AM-V0036M	AM-V0039M	AM-V0030M
	hmAG407	Monomer	407	498	AM-V0504M				
Kusabira -Orange	KO1	Dimer	548	561	AM-V0041M				
	mKO1	Monomer	548	559	AM-V0051M	AM-V0052M	AM-V0053M		
	mKO2	Monomer	551	565	AM-V0141M				
	hKO1	Dimer	548	561	AM-V0044M	AM-V0045M	AM-V0046M		
	hmKO1	Monomer	548	559	AM-V0054M	AM-V0055M	AM-V0056M	AM-V0059M	AM-V0050M
	hmKO2	Monomer	551	565		AM-V0145M	AM-V0146M	AM-V0149M	AM-V0140M
Keima-Red	dKeima570	Dimer	440	570	AM-V0121M				
	hdKeima570	Dimer	440	570	AM-V0124M			AM-V0129M	AM-V0120M
	dKeima-Red	Dimer	440	616	AM-V0101M				
	mKeima-Red	Monomer	440	620	AM-V0091M		AM-V0093M		
	hdKeima-Red	Dimer	440	616	AM-V0104M			AM-V0109M	AM-V0100M
	hmKeima-Red	Monomer	440	620	AM-V0094M			AM-V0099M	AM-V0090M
Dronpa Green	DG1	Monomer	503	518	AM-V0071M	AM-V0072M	AM-V0073M		
	hDG1	Monomer	503	518				AM-V0079M	AM-V0070M
	DG3	Monomer	491	514	AM-V0131M				
Kaede	Kaede	Tetramer	508/572	518/580	AM-V0011M	AM-V0012M	AM-V0013M		
Kikume Green-Red	KikGR1	Tetramer	507/583	517/593	AM-V0081M	AM-V0082M	AM-V0083M		
	mKikGR1	Monomer	505/580	517/591	AM-V0151M				
	hKikGR1	Tetramer	507/583	517/593	AM-V0084M	AM-V0085M	AM-V0086M	AM-V0089M	AM-V0080M
	hmKikGR1	Monomer	505/580	517/591				AM-V0159M	AM-V0150M

S1: Vectors for subcloning
MC1, MN1: Expression vectors
MCLinker, MNLiner: Expression vectors with flexible linker
h: Humanized-codon
m: Monomer
d: Dimer

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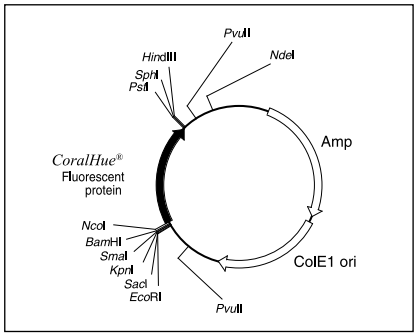
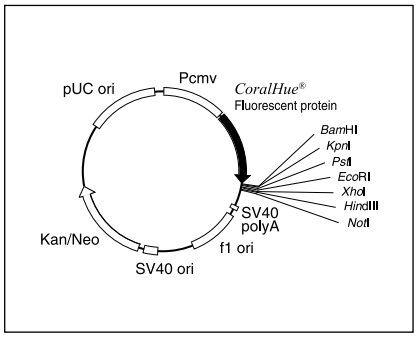
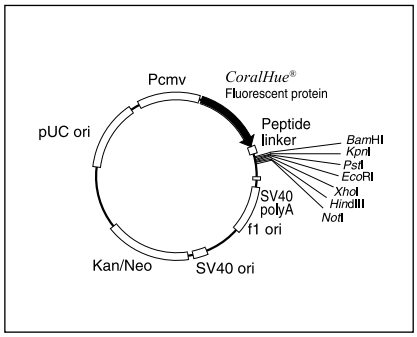
S1



MC1



MCLinker



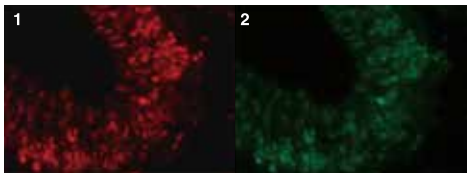
Anti-Fluorecsent Protein Antibodies

Code no.	Products	Clone	Volume	Applications	Western Blot cross reactivity
M102-3M	Anti-monomeric Azami-Green1 mAb	2F11	100 μg/100 μL	WB	mAG1
PM052M	Anti-monomeric Azami-Green1 pAb	Polyclonal	100 μL	WB, IP, IC, IH	mAG1
M103-3M	Anti-Azami-Green mAb	3D10	100 μg/100 μL	IP	
PM011M	Anti-Azami-Green pAb	Polyclonal	100 μL	WB	AG, mAG1
M117-3M	Anti-Dronpa-Green mAb	4D12	100 μg/100 μL	WB	DG1, DG3
M118-3M	Anti-Dronpa-Green mAb	2F6	100 μg/100 μL	IP	
M106-3M	Anti-Kaede mAb	2F4	100 μg/100 μL	IP	
M125-3M	Anti-Kaede mAb	3B1	100 μg/100 μL	WB	
PM012M	Anti-Kaede pAb	Polyclonal	100 μL	WB	
M126-3M	Anti-monomeric Keima-Red mAb	2F7	100 μg/100 μL	WB	mKeima-Red
M127-3M	Anti-Keima-Red mAb	3C9	100 μg/100 μL	IP	
M182-3M	Anti-Keima-Red mAb	1C3	100 μg/100 μL	WB	
M128-3M	Anti-Kikume Green-Red mAb	5B3	100 μg/100 μL	WB	KikGR, mKikGR
M129-3M	Anti-Kikume Green-Red mAb	2D3	100 μg/100 μL	IP	
M104-3M	Anti-monomeric Kusabira-Orange1 mAb	1H7	100 μg/100 μL	WB	mKO1, mKO2, mKG, mKG-O, mKOkappa
M105-3M	Anti-monomeric Kusabira-Orange1 mAb	2G9	100 μg/100 μL	IP	
M168-3M	Anti-monomeric Kusabira-Orange2 mAb	3B3	100 μg/100 μL	WB, IP, IC, IH	mKO2, mKG, mKG-O, mKOkappa
PM051M	Anti-monomeric Kusabira-Orange2 pAb	Polyclonal	100 μL	WB, IP, IC, IH	KO1,mKO1,mKO2,mKG,mKG-O,mKOkappa
M116-3M	Anti-Midoriishi-Cyan mAb	2C1	100 μg/100 μL	IP	
M130-3M	Anti-Midoriishi-Cyan mAb	5B7	100 μg/100 μL	WB	MiCy, mMiCy
M148-3M	Anti-monomeric Kusabira-Green N-terminal Fragment mAb	1E6	100 μg/100 μL	WB	mKO1, mKO2, mKG
M149-3M	Anti-monomeric Kusabira-Green C-terminal Fragment mAb	21B10	100 μg/100 μL	WB	mKO2, mKG

WB: Western blotting, IP: Immunoprecipitation, IC: Immunocytochemistry, IH: Immunohistochemistry

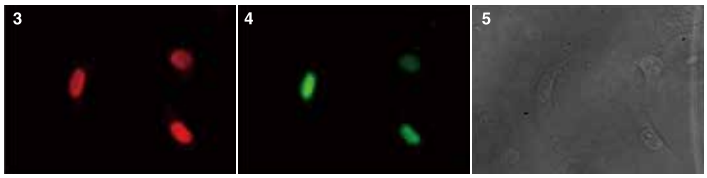
Anti-monomeric Azami-Green1 pAb (Code No. PM052M)

Immunohistochemistry



Immunohistochemical detection of mAG1 on frozen section of B6.Cg-Tg (Fucci) 504Bsi mouse embryonic brain (E13) with PM052M (1) and Fucci-S/G_2/M Green own fluorescence (2).

Immunocytochemistry

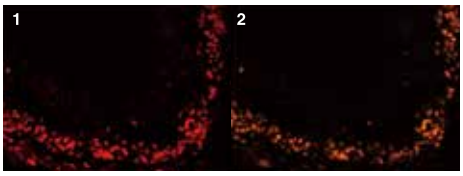


Immunocytochemical detection of mAG1 in Fucci-S/G_2/M Green transfected HeLa cells with PM052M.

3: Anti-mAG1 (PM052M)
4: Fucci-S/G_2/M Green
5: Transmission light

Anti-monomeric Kusabira-Orange2 mAb (Code No. M168-3M)

Immunohistochemistry



Immunohistochemical detection of mKO2 on frozen section of B6. Cg-Tg (Fucci) 596Bsi mouse embryonic brain (E12) with M168-3M (1) and Fucci-G_1 Orange own fluorescence (2).

Immunocytochemistry

Immunocytochemical detection of mKO2 in Fucci-G_1 Orange transfected HeLa cells with M168-3M.

3: Anti-mKO2 (M168-3M)
4: Fucci-G_1 Orange
5: Transmission light

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