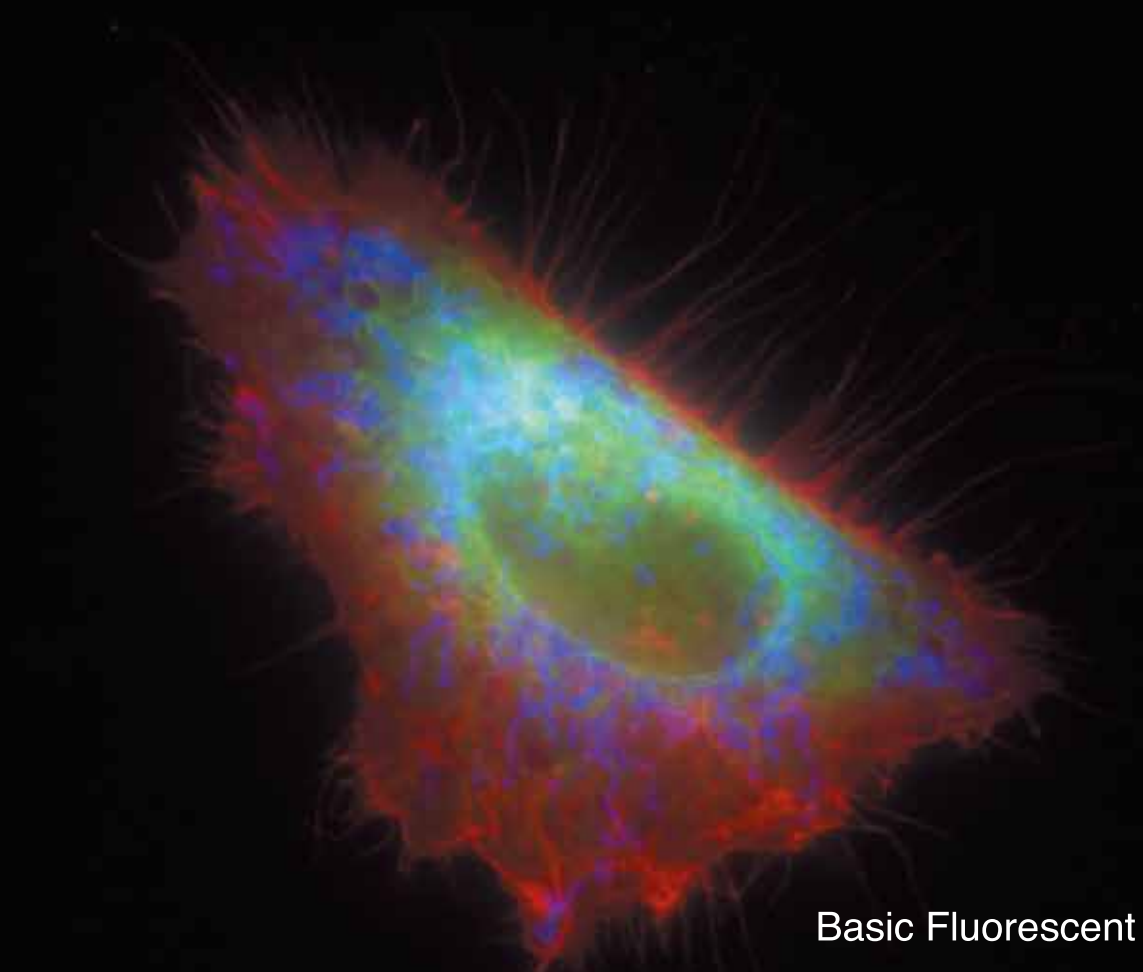


Amalgam *Fluorescent Proteins*



Basic Fluorescent Proteins

Photoconvertible Fluorescent Proteins

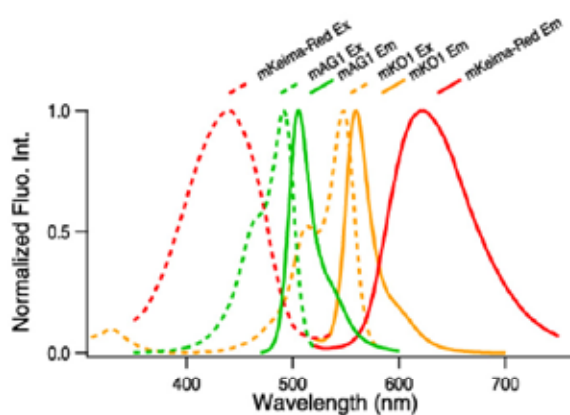
Protein-Protein Interaction Detection System

Advanced Fluorescent Indicator

Antibodies

Contents

<i>CoralHue</i>[®] Basic Fluorescent Proteins	3
• Midoriishi-Cyan	
• Umikinoko-Green	
• Azami-Green	
• Kusabira-Orange	
• Keima570	
• Keima-Red	
• Organelle targeting vectors	
<i>CoralHue</i>[®] Photoconvertible Fluorescent Proteins	11
• Dronpa-Green	
• Kaede	
• Kikume Green-Red	
Protein-Protein Interaction Detection System	14
• <i>CoralHue</i>[®] Fluo-Chase Kit	
Advanced Fluorescent Indicator	15
• Fucci (fluorescent ubiquitination-based cell cycle indicator)	
Antibodies	16
Product List	18
References	



Multicolor labeling of mammalian cells

An image of the HeLa cell with mKO1 localized on the plasma membrane (red), mAG1 in the endoplasmic reticulum (green) and mKeima-Red in the mitochondria (blue).

Filters and mirrors

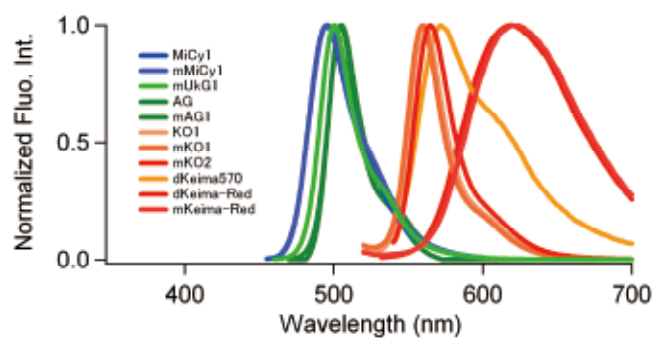
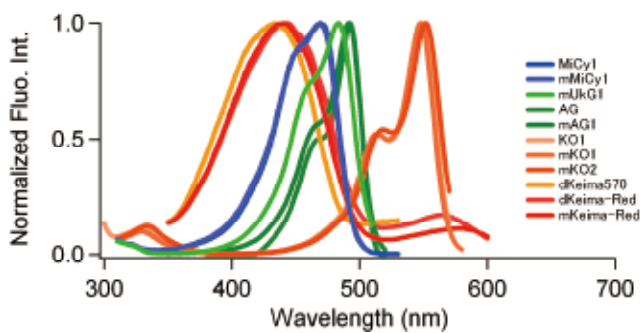
mKeima-Red Ex : 440AF21, Em : 610ALP, DM : 590DRLP

mAG1 Ex : BP460-480HQ, Em : BA495-540HQ, DM : DM485

mKO1 Ex : BP520-540HQ, Em : BA555-600HQ, DM : DM545HQ

CoralHue® Basic fluorescent proteins

Amalgam offers various fluorescent colors, from cyan to red, for labeling cells, subcellular structures and proteins, without cytotoxicity. Stable transformant cells and transgenic animals can also be established with some of our basic fluorescent proteins.



Products Name	Color	Excit./Emiss. Maxima (nm)	Oligomerization	*Brightness
MiCy1: Midoriishi-Cyan1	Cyan	472/495	Dimer	24.5
mMiCy1: monomeric Midoriishi-Cyan1	Cyan	470/496	Monomer	15.5
mUkG1: monomeric Umikinoko-Green1	Green	483/499	Monomer	43.2
AG: Azami-Green	Green	492/505	Tetramer	48.4
mAG1: monomeric Azami-Green1	Green	492/505	Monomer	40.7
KO1: Kusabira-Orange1	Orange	548/561	Dimer	33.2
mKO1: monomeric Kusabira-Orange1	Orange	548/559	Monomer	31.0
mKO2: monomeric Kusabira-Orange2	Orange	551/565	Monomer	39.6
dKeima570	Orange	440/570	Dimer	2.1
dKeima-Red	Red	440/616	Dimer	7.6
mKeima-Red: monomeric Keima-Red	Red	440/620	Monomer	3.5

* Brightness: Molar Extinction Coefficient x Fluorescence Quantum Yield / 1000

Midoriishi-Cyan: MiCy

- FRET donor with highly fluorescent quantum yield
- Cell, subcellular structure, or protein labeling

The fluorescent protein Midoriishi-Cyan gene was isolated from the stony coral *Acropora* sp.(Midorii-shi in Japanese).

CoralHue® Midoriishi-Cyan 1 (MiCy1) absorbs light maximally at 472 nm and emits cyan light at 495 nm. It rapidly matures to form a bright dimeric complex. So it can be used to mark individual cells or to report gene expression without the problem of protein aggregation. **CoralHue®** mMiCy1 is a monomeric version of MiCy1, and can be used to label proteins or subcellular structures.

These fluorescent proteins are suitable as donor for fluorescence resonance energy transfer (FRET) because of highly fluorescent quantum yield.

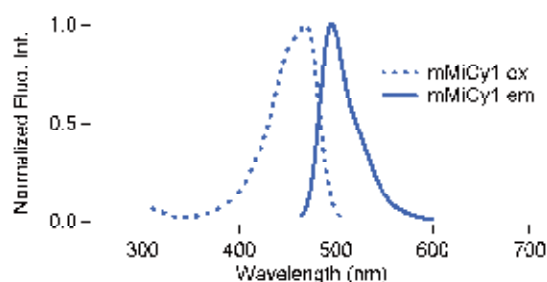
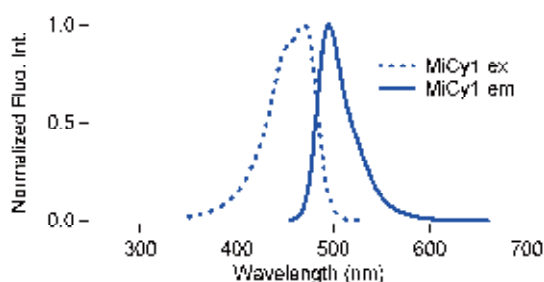


Stony coral "Midori-ishi" (*Acropora* sp.).



CoralHue® MiCy1 expression in HeLa cells.

Fluorescent properties



CHARACTERISTIC	MiCy1	mMiCy1
Oligomerization	dimer	monomer
Number of amino acid	232	232
Excit./Emiss. Maxima (nm)	472/495	470/496
Molar Extinction Coefficient (M ⁻¹ cm ⁻¹)	27,250 (472 nm)	22,150 (470 nm)
Fluorescence Quantum Yield	0.90	0.70
Brightness ^{*1}	24.5	15.5
pH sensitivity	pKa=6.6	pKa=7.0
Cytotoxicity ^{*2}	No	No

^{*1}Brightness: Molar Extinction Coefficient × Fluorescence Quantum Yield / 1000

^{*2}Toxicity when expressed in HeLa cells

Umikinoko-Green: UkG

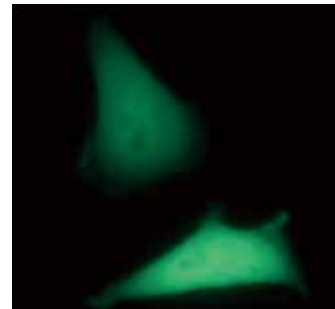
- Bright green fluorescence
- FRET donor with high pH stability

The fluorescent protein Umikinoko-Green (UkG) gene was isolated from the soft coral *Sarcophyton* sp. (Umi-kinoko in Japanese).

UkG rapidly matures to form a dimeric complex. *CoralHue*[®] monomeric Umikinoko-Green (mUkG1) maintains the brightness and pH stability of parent protein UkG. mUkG1 absorbs light maximally at 483 nm and emits green light at 499 nm. mUkG1 exhibits brilliant fluorescence and has extremely high pH stability. mUkG1 can be used to label proteins, subcellular structures or for FRET analysis.

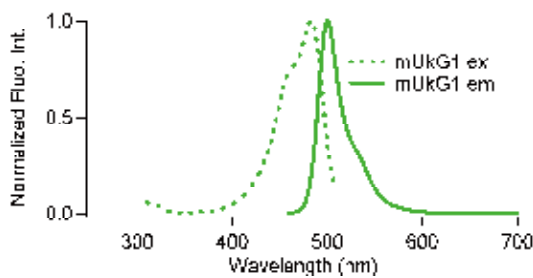


Soft coral "Umi-kinoko" (*Sarcophyton* sp.).



CoralHue[®] mUkG1 expression in HeLa cells.

Fluorescent properties



CHARACTERISTIC	mUkG1
Oligomerization	monomer
Number of amino acid	227
Excit./Emiss. Maxima (nm)	483/499
Molar Extinction Coefficient (M ⁻¹ cm ⁻¹)	60,000 (483 nm)
Fluorescence Quantum Yield	0.72
Brightness ^{*1}	43.2
pH sensitivity	pKa=5.2
Cytotoxicity ^{*2}	No

^{*1}Brightness: Molar Extinction Coefficient × Fluorescence Quantum Yield / 1000

^{*2}Toxicity when expressed in HeLa cells

Azami-Green: AG

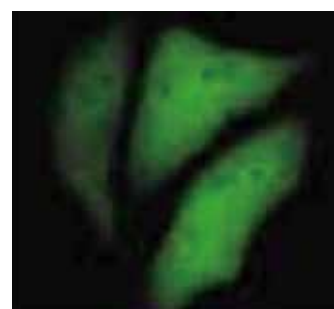
- Bright green fluorescence
- High pH stability
- Monomer/tetramer

CoralHue[®] Azami-Green (AG), cloned from the stony coral *Galaxea fascicularis* (Azami-sango in Japanese), absorbs light maximally at 492 nm and emits green light at 505 nm. AG is stable in the biological pH range and does not show a significant loss of fluorescent signal, making it advantageous over other fluorescent proteins such as EGFP. And AG also matures rapidly to form tetramers that are brighter than EGFP.

CoralHue[®] monomeric Azami-Green (mAG1) maintains the brightness and pH stability of the parent protein AG. mAG1 can be used for labeling proteins or subcellular structures.

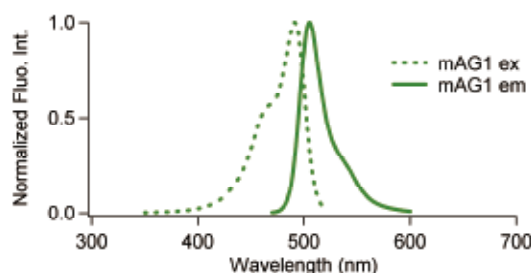
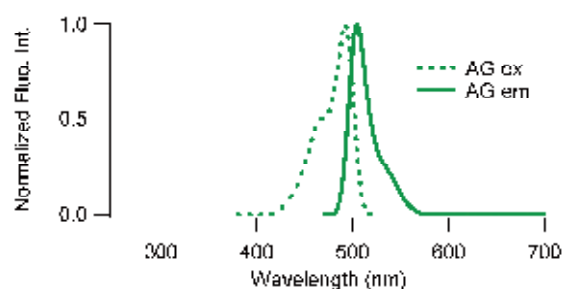


Recombinant *CoralHue*[®] Azami-Green protein exposed to fluorescent light (left) and a UV transilluminator (right).



CoralHue[®] AG expression in HeLa cells.

Fluorescent properties



CHARACTERISTIC	AG	mAG1
Oligomerization	Tetramer	Monomer
Number of amino acid	225	225
Excit./Emiss. Maxima (nm)	492/505	492/505
Molar Extinction Coefficient (M ⁻¹ cm ⁻¹)	72,300 (492 nm)	55,500 (492 nm)
Fluorescence Quantum Yield	0.67	0.74
Brightness ^{*1}	48.4	41.0
pH sensitivity	pKa<5.0	pKa=5.8
Cytotoxicity ^{*2}	No	No

^{*1}Brightness: Molar Extinction Coefficient × Fluorescence Quantum Yield / 1000

^{*2}Toxicity when expressed in HeLa cells

Kusabira-Orange: KO

- Bright orange fluorescence
- High pH stability
- Monomer/dimer

CoralHue® Kusabira-Orange (KO1), from the stony coral *Fungia concinna* (Kusabira-ishi in Japanese), absorbs light maximally at 548 nm and emits orange light at 561 nm. KO1 rapidly matures to form a fluorescent dimeric complex. KO1 can be used to mark cells or to report gene expression without problems stemming from protein aggregation.

CoralHue® monomeric Kusabira Orange (mKO1) maintains the brilliance and pH stability of the parent protein. mKO1 can be used to label proteins or subcellular structures or for FRET analysis.

CoralHue® mKO2 is a mutant of mKO1 that features rapid maturation. mKO2 can be used to label proteins or subcellular structures or for reporter assays.

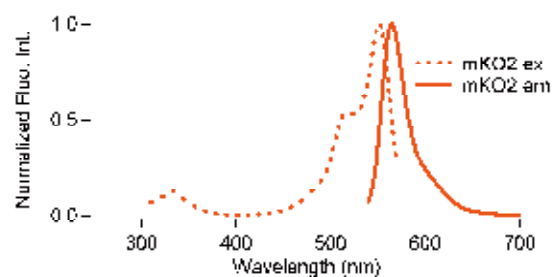
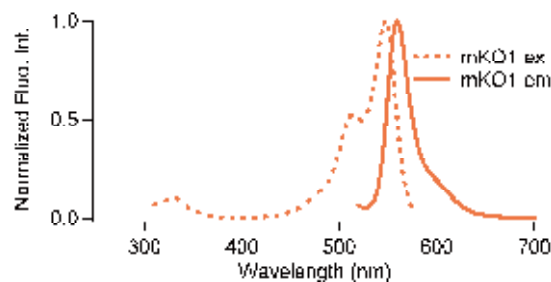
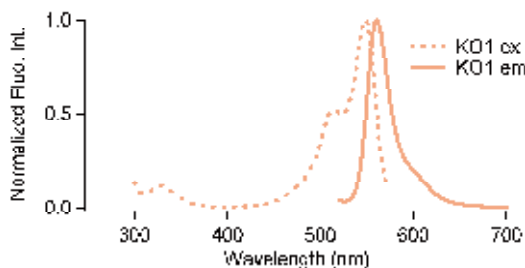


Recombinant **CoralHue®** Kusabira-Orange protein exposed to fluorescent light (left) and UV transilluminator (right).



Kusabira-Orange 1 TG pig
Provided by Dr. Nagashima at Meiji University Cloning and Stem Cells. (2008) 10: 313-324.

Fluorescent properties



CHARACTERISTIC	KO1	mKO1	mKO2
Oligomerization	Dimer	Monomer	Monomer
Number of amino acid	218	218	218
Excit./Emiss. Maxima (nm)	548/561	548/559	551/565
Molar Extinction Coefficient (M ⁻¹ cm ⁻¹)	73,700 (548 nm)	51,600 (548 nm)	63,800 (551 nm)
Fluorescence Quantum Yield	0.45	0.60	0.62
Brightness ^{*1}	33.2	31.0	39.6
pH sensitivity	pKa<5.0	pKa=5.0	pKa=5.5
Cytotoxicity ^{*2}	Not observed	Not observed	Not observed
Resistance to PFA fixation	Not tested	Not tested	+++

^{*1}Brightness: Molar Extinction Coefficient × Fluorescence Quantum Yield / 1000

^{*2}Toxicity when expressed in HeLa cells

Keima570

- Large Stokes shift
- Unique spectrum

CoralHue® dKeima570, originally cloned from the stony coral *Montipora* sp. (Komon-sango in Japanese), absorbs light maximally at 440 nm and emits orange-red light at 570 nm. Because of the extremely large Stokes shift (130 nm) of **CoralHue®** dKeima570, the maximum fluorescence can be obtained by the maximum excitation without sacrificing either excitation or fluorescence.

Although several fluorescent proteins have a large Stokes shift, they have green fluorescence as a result of excitation with UV light at around 380 nm. However, the use of such toxic UV light is not suitable for observation in living organisms. Keima is the first red fluorescent protein having a large Stokes shift.

The combination of orange-red emission, a large Stokes shift, stability at 37 °C in eukaryotic cells, and being dimeric make **CoralHue®** dKeima570 a superb reporter protein for labeling subcellular structures in multicolor fluorescence analyses. The orange-red fluorescence is stable under normal aerobic conditions.

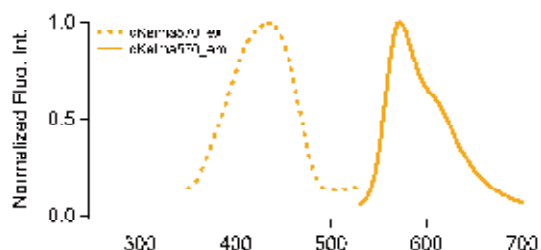


Stony coral "Komon-Sango" (*Montipora* sp.).



CoralHue® dKeima570 expression in HeLa cells.

Fluorescent properties



CHARACTERISTIC	dKeima570
Oligomerization	Dimer
Number of amino acid	222
Excit./Emiss. Maxima (nm)	440/570
Molar Extinction Coefficient (M ⁻¹ cm ⁻¹)	14,000 (440 nm)
Fluorescence Quantum Yield	0.15
Brightness ^{*1}	2.1
pH sensitivity	pKa=6.5
Cytotoxicity ^{*2}	No

^{*1}Brightness: Molar Extinction Coefficient × Fluorescence Quantum Yield / 1000

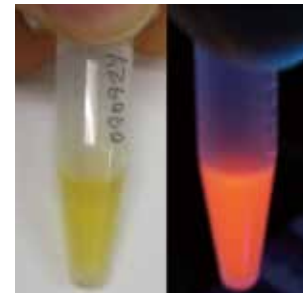
^{*2}Toxicity when expressed in HeLa cells

Keima-Red

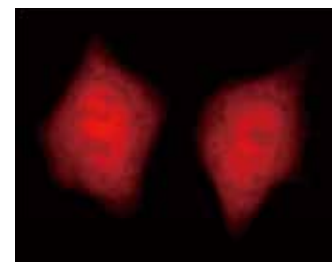
- Large Stokes shift
- For use with simultaneous multicolor imaging
- For use with single laser line FCCS

CoralHue[®] dimeric Keima-Red (dKeima-Red) and *CoralHue*[®] monomeric Keima-Red (mKeima-Red) are red fluorescent proteins with extremely large Stokes shift. They absorb light maximally at 440 nm and emit red light at 616 nm and 620 nm, respectively. There are no other fluorescent proteins with this unique fluorescence. Because of this characteristic, they are excited by a very short wavelength but emit a long wavelength. Keima is named after a shogi (Japanese chess) piece that can move in the hopping manner, similar to the knight in the game of chess.

The large Stokes shift property of Keima-Red allows effective applications such as for single wavelength excitation simultaneous multi-color imaging and single laser line FCCS.

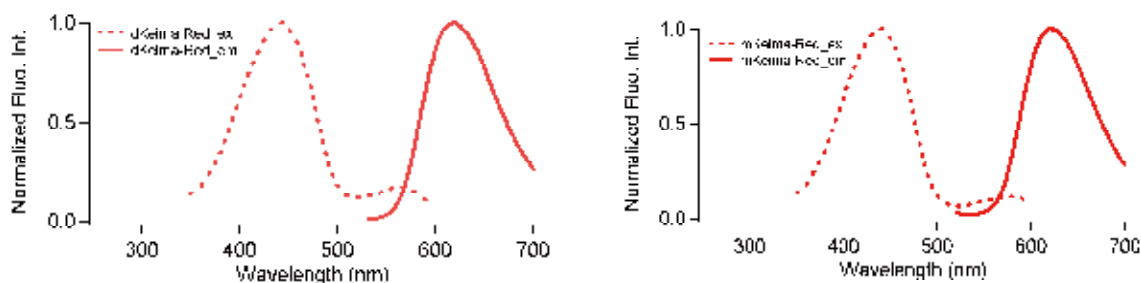


Recombinant *CoralHue*[®] Keima-Red protein exposed to fluorescent light (left) and a UV transilluminator (right).



CoralHue[®] mKeima-Red expression in HeLa cells.

Fluorescent properties



CHARACTERISTIC	dKeima-Red	mKeima-Red
Oligomerization	Dimer	Monomer
Number of amino acid	222	222
Excit./Emiss. Maxima (nm)	440/616	440/620
Molar Extinction Coefficient (M ⁻¹ cm ⁻¹)	24,600 (440 nm)	14,400 (440 nm)
Fluorescence Quantum Yield	0.31	0.24
Brightness ^{*1}	7.6	3.5
pH sensitivity	pKa=6.5	pKa=6.5
Cytotoxicity ^{*2}	No	No

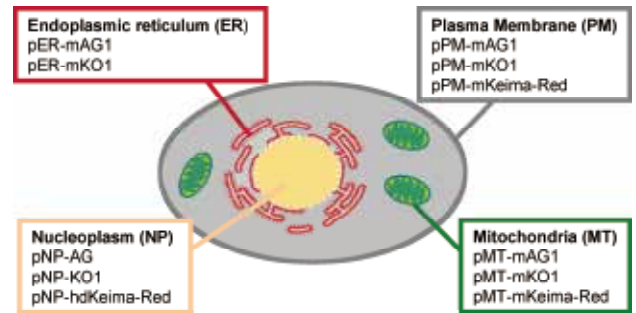
^{*1}Brightness: Molar Extinction Coefficient × Fluorescence Quantum Yield / 1000

^{*2}Toxicity when expressed in HeLa cells

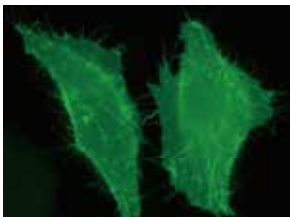
Organelle targeting vectors

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

CoralHue® Organelle targeting vectors allowing to visualize subcellular structures. These vectors encode fusions of fluorescent protein variants and localization signals. It can be used for fluorescent labeling of subcellular structure in living cells.

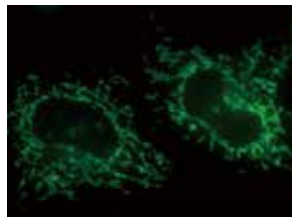


Plasma membrane (PM)



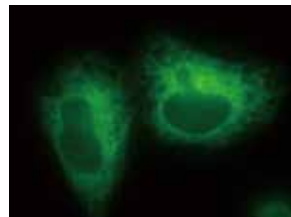
CoralHue® pPM-mAG1

Mitochondria (MT)



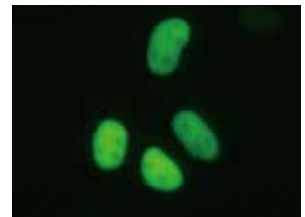
CoralHue® pMT-mAG1

Endoplasmic reticulum (ER)

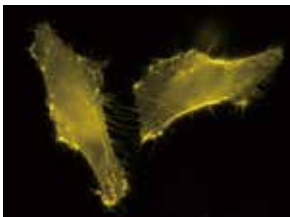


CoralHue® pER-mAG1

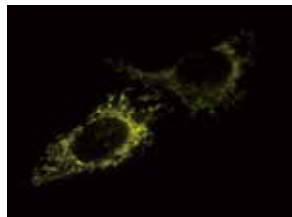
Nucleoplasm (NP)



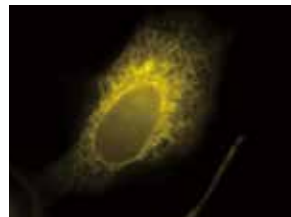
CoralHue® pNP-AG



CoralHue® pPM-mKO1



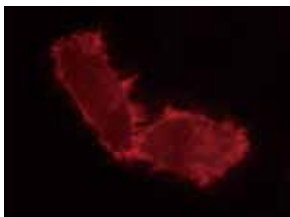
CoralHue® pMT-mKO1



CoralHue® pER-mKO1



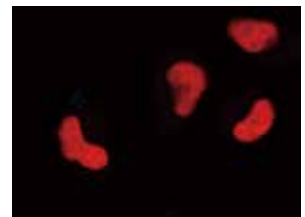
CoralHue® pNP-KO1



CoralHue® pPM-mKeima-Red

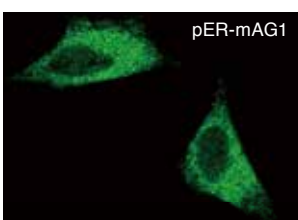


CoralHue® pMT-mKeima-Red

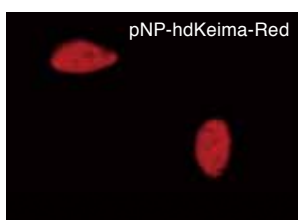


CoralHue® pNP-hdKeima-Red

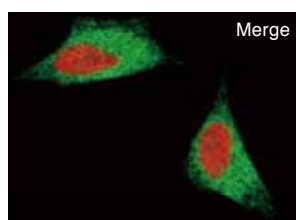
Dual-labeling cells with *CoralHue®* fluorescent proteins



pER-mAG1



pNP-hdKeima-Red



Merge

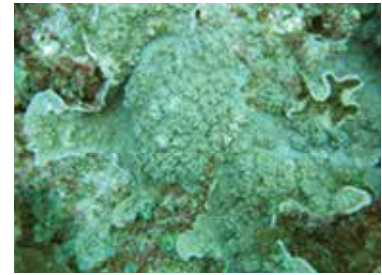
HeLa cells were transiently transfected with vectors encoding *CoralHue®* pER-mAG1 and *CoralHue®* pNP-hdKeima-Red. Cells were imaged on an inverted confocal laser scanning microscope (Carl Zeiss, LSM510).

Dronpa-Green: DG

- Photoswitchable fluorescent protein
- Reversible “on-off” switching
- Useful for molecular dynamics investigation

The fluorescent protein Dronpa gene was originally cloned from stony coral *Echinophyllia* sp. (Kikka-sango in Japanese).

CoralHue® Dronpa-Green1 (DG1) is a monomeric green fluorescent protein that is photochromic: its fluorescence is reversible, with "on-off" switching by demand through exposure to different wavelengths of light. DG1 bleaches immediately following strong excitation at ~500 nm. Bleached DG1 completely regains its bright green fluorescence after irradiation at ~400 nm. This unique property of DG1 is useful for repeated measurements of mobility dynamics (e.g. diffusion, transport) of fluorescent-labeled molecules in living cells.

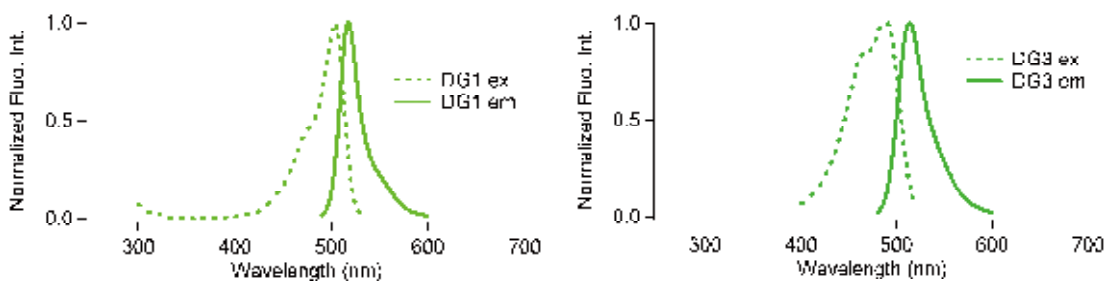


Stony coral "Kikka-sango" (*Echinophyllia* sp.).



CoralHue® DG1 expressing HeLa cells.

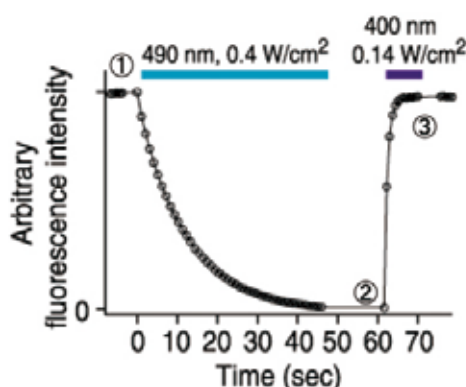
Fluorescent properties



CHARACTERISTIC	DG1	DG3
Oligomerization	Monomer	Monomer
Number of amino acid	225	225
Excit./Emiss. Maxima (nm)	503/518	491/514
Molar Extinction Coefficient (M ⁻¹ cm ⁻¹)	95,000 (503 nm)	58,000 (487 nm)
Fluorescence Quantum Yield	0.85	0.28
Brightness ^{*1}	80.7	16.2
pH sensitivity	pKa=5.0	pKa=5.0
Cytotoxicity ^{*2}	No	No

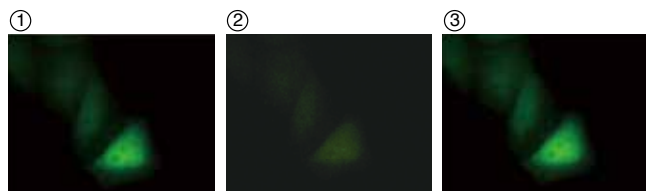
^{*1}Brightness: Molar Extinction Coefficient × Fluorescence Quantum Yield / 1000

^{*2}Toxicity when expressed in HeLa cells



Photochromic properties of Dronpa

Dronpa was transfected into HeLa cells followed by fixation and the time course of fluorescence was monitored. Photoactivation of DG1 required much less photon energy than did photobleaching, with respective quantum yields of 0.37 (ΦPA) and 0.00032 (ΦPB).



Ando, R., Mizuno, H. and Miyawaki, A.(2004) Science 306, 1370-1373.

Kaede

- Photoconvertible fluorescent protein
- Optical marking

The fluorescent protein Kaede gene was isolated from the stony coral *Trachyphyllia geoffroyi* (Hiyu-sango in Japanese). Kaede means maple leaf in Japanese.

CoralHue® Kaede protein emits bright green fluorescence that can irreversibly convert to red.

The red fluorescence is comparable in intensity to the green and is stable under usual aerobic conditions. The green-to-red conversion is highly sensitive to irradiation with UV or violet light (350-410 nm). Maximal illumination results in a 2,000-fold increase in the ratio of red-to-green signaling. The excitation lights used to elicit red or green fluorescence do not induce the photoconversion.



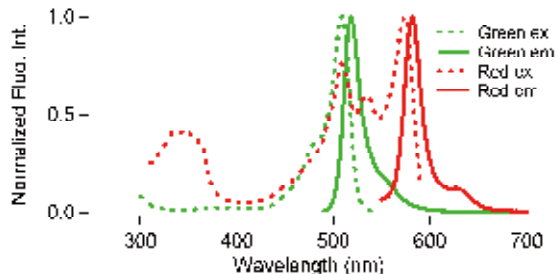
Stony coral "Hiyu-Sango" (*Trachyphyllia geoffroyi*).



Kaede TG mice

Provided by Dr. Miwa at Tsukuba University
Proc Natl Acad Sci U S A. (2008) 105: 10871-10876

Fluorescent properties



CHARACTERISTIC	Kaede	
		(photoconverted)
Oligomerization	Tetramer	Tetramer
Number of amino acid	225	225
Excit./Emiss. Maxima (nm)	508/518	572/580
Molar Extinction Coefficient (M ⁻¹ cm ⁻¹)	98,800 (508 nm)	60,400 (572 nm)
Fluorescence Quantum Yield	0.88	0.33
Brightness ^{*1}	86.9	19.9
pH sensitivity	pKa=5.6	pKa=5.6
Cytotoxicity ^{*2}	No	No

^{*1}Brightness: Molar Extinction Coefficient × Fluorescence Quantum Yield / 1000

^{*2}Toxicity when expressed in HeLa cells

Kikume Green-Red: KikGR

- Photoconvertible fluorescent protein
- Monomer / tetramer

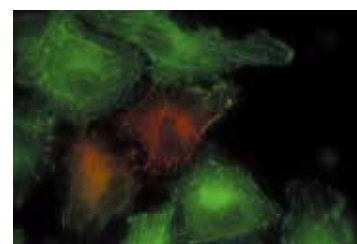
The fluorescent protein Kikume Green gene was isolated from the stony coral *Favia fавus* (Kikume-ishi in Japanese).

CoralHue® Kikume Green-Red1 (KikGR1) protein emits bright green fluorescence that can be irreversibly converted to red. KikGR1 can be photoconverted by irradiation with UV or violet light (350-410 nm). Photoconverted KikGR1 possesses excitation/emission maxima at 583/593 nm. KikGR1 contains a His62-Tyr63-Gly64 tripeptide sequence which forms a green chromophore that can be photoconverted to a red one via formal beta-elimination and subsequent extension of the π -conjugated system.

CoralHue® monomeric Kikume Green-Red (mKikGR1) maintains the brightness of the parent protein KikGR1. mKikGR1 can be used for labeling proteins or subcellular structures.



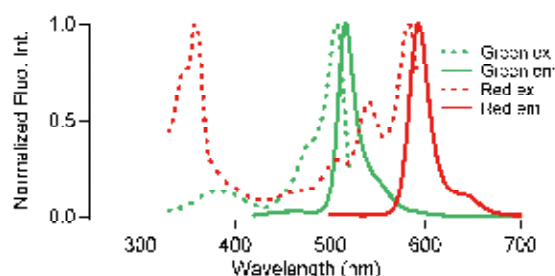
CoralHue® KikGR1 expression in neurons.
Provided by Dr. Tsutsui and Dr. Miyawaki
Laboratory for Cell Function and Dynamics,
BSI, RIKEN



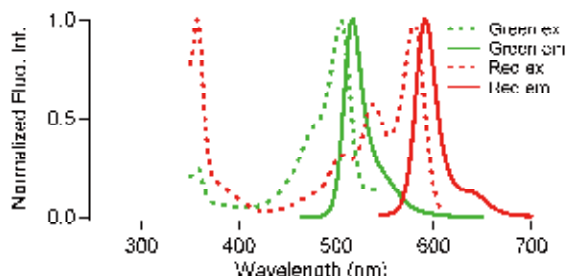
HeLa cells expressed hKikGR1 fusion β -actin with a flexible linker followed by green-to-red photoconversion with UV light.

Fluorescent properties

CoralHue® KikGR1



CoralHue® mKikGR1



CHARACTERISTIC	KikGR1		mKikGR1	
		(photoconverted)		(photoconverted)
Oligomerization	Tetramer	Tetramer	Monomer	Monomer
Number of amino acid	225	225	233	223
Excit./Emiss. Maxima (nm)	507/517	583/593	505/517	580/591
Molar Extinction Coefficient ($M^{-1}cm^{-1}$)	53,700 (507 nm)	35,100 (583 nm)	47,100 (504 nm)	21,750 (579 nm)
Fluorescence Quantum Yield	0.70	0.65	0.53	0.64
Brightness ¹	37.6	22.8	25.0	13.9
pH sensitivity	pKa=7.8	pKa=5.5	pKa=6.5	pKa=5.2
Cytotoxicity ²	No	No	No	No

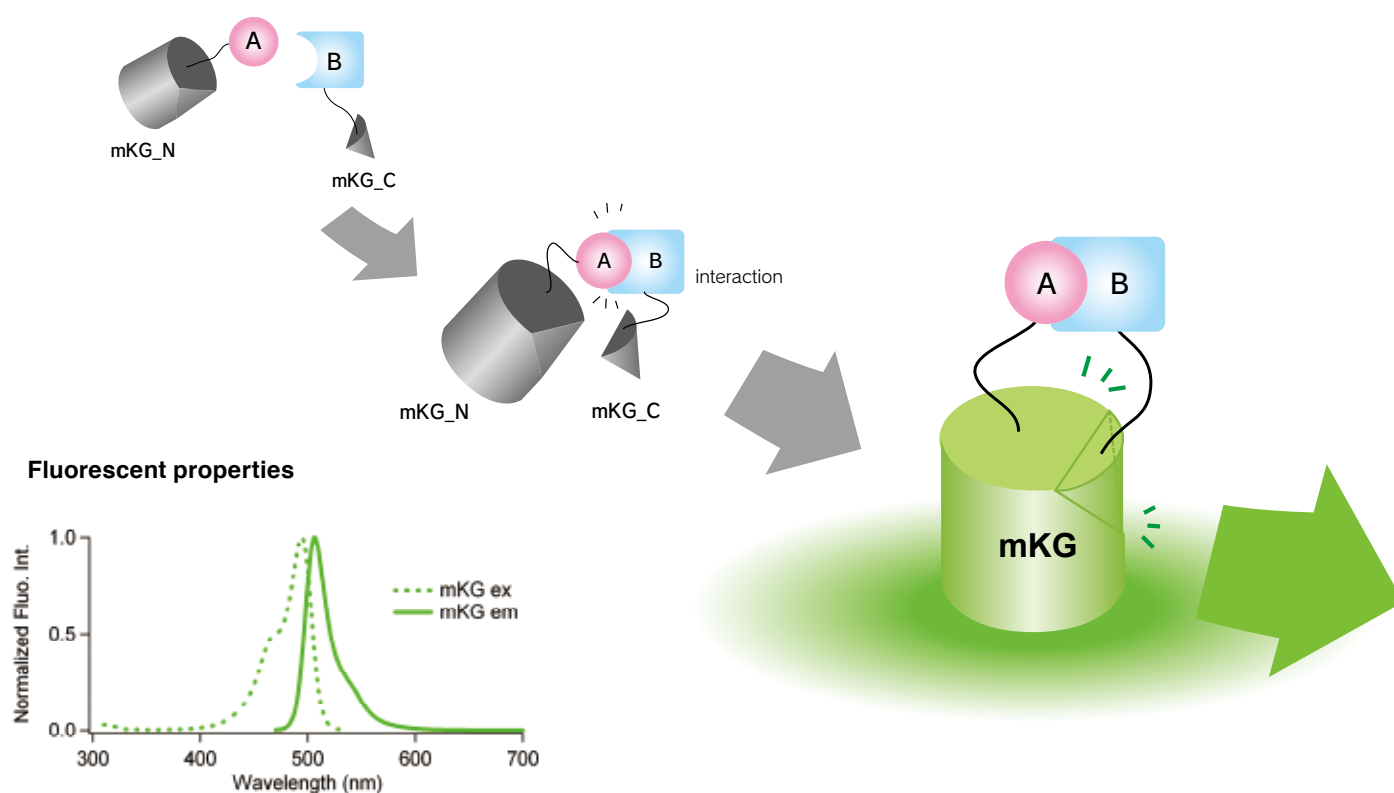
¹Brightness: Molar Extinction Coefficient \times Fluorescence Quantum Yield / 1000

²Toxicity when expressed in HeLa cells

CoralHue[®] Fluo-chase Kit

- Protein-protein interaction (PPI) analysis
- Direct visualization of PPIs in living cells
- Easy construction

CoralHue[®] Fluo-chase Kit is based on the protein fragment complementation assay using our proprietary fluorescent protein, *CoralHue[®]* monomeric Kusabira Green (mKG). mKG is split into two inactive fragments, mKG_N and mKG_C, and do not emit fluorescence on their own. With the *CoralHue[®] Fluo-chase Kit* the protein of interest (A) is expressed as a fusion protein to the mKG_N (A-mKG_N) and the mKG_C is fused to the second protein of interest (B), (B-mKG_C). Both vectors encoding mKG fragment fusion protein are co-introduced into mammalian cells. When the mKG fragments are in close proximity due to the interaction of A and B, the mKG fragments form a beta-barrel structure and emit green fluorescence.



CHARACTERISTIC	Kusabira-Green
Fluorescence color	Green
Oligomerization	monomer
Excitation max (NM)	494
Emission max (nm)	506
Molar Extinction Coefficient (M ⁻¹ cm ⁻¹)	63,200 (494 nm)
Fluorescence Quantum Yield	0.57
Brightness *	36.0
pH sensitivity in fluorescence	pKa=6.1
Molecular weight (kDa)	24.5

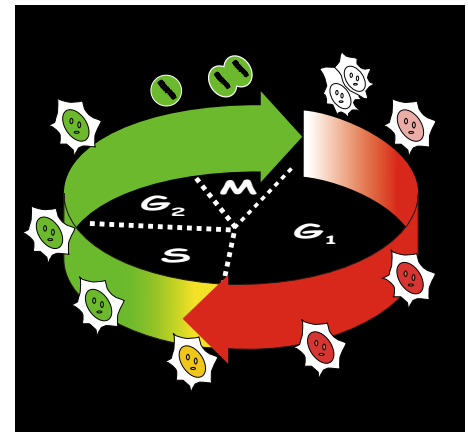
* Brightness: Molar Extinction Coefficient × Fluorescence Quantum Yield / 1000

Fucci

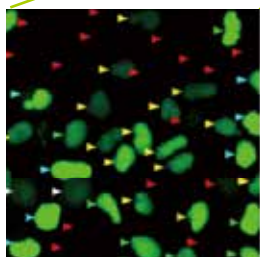
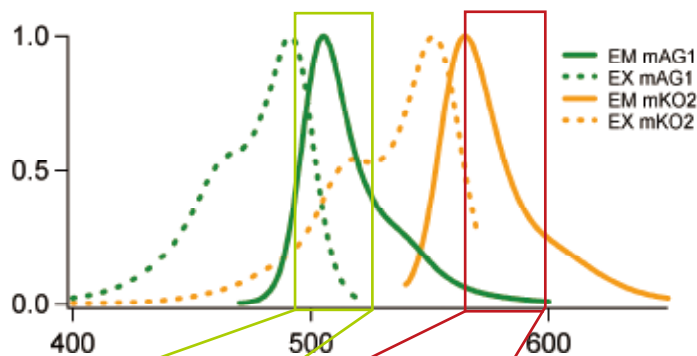
Fluorescent Ubiquitination-based Cell Cycle Indicator

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

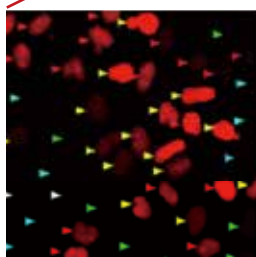
Fluorescent ubiquitination-based cell cycle indicator (Fucci) is a sophisticated technology which can easily determine G₁ and/or S/G₂/M phases of the cell cycle. The technology analyzes living cells in a spatio-temporal manner using a dual color scheme of orange and green. Fucci was successfully established by intelligently utilizing ubiquitin-proteasome protein degradation system.



Schematic representation of Fucci cell-cycle labeling Fucci-G₁ Orange labels G₁ phase nuclei in orange. Fucci-S/G₂/M Green labels S/G₂/M phases nuclei in green.



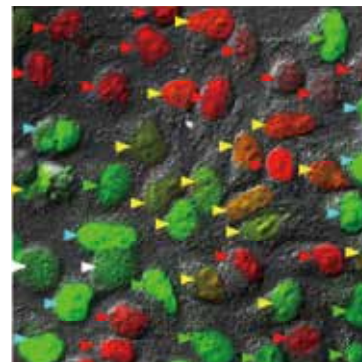
Fucci-S/G₂/M Green



Fucci-G₁ Orange

Fluorescence characteristics

The technology was established by using Amalgaam's proprietary fluorescent proteins: mAG1 (monomeric Azami-Green1) and mKO2 (monomeric Kusabira-Orange2). Both are bright fluorescent proteins and mature rapidly. The graph above shows excitation (dotted line) and emission (solid line) spectra of these proteins.



Merge + DIC

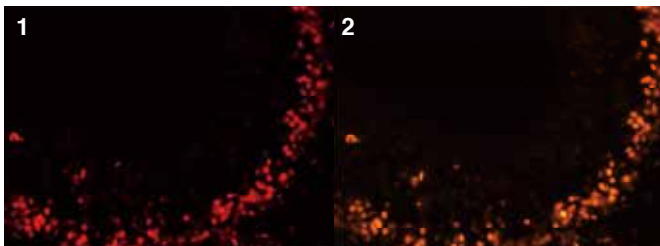
Provided by Dr. Sakaue-Sawano at RIKEN. Cell (2008) 132:487-98.



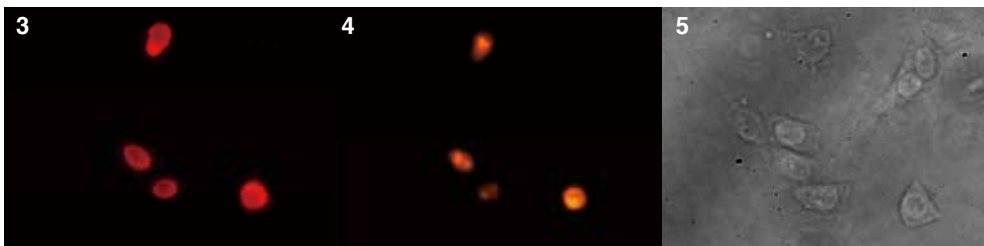
Antibodies

Anti-monomeric Kusabira-Orange 2 (polyclonal antibody): code No. PM051

PM051 is available for immunostaining of “Fucci-G₁ Orange” (Fucci; Fluorescent Ubiquitination-based Cell Cycle Indicator). Fucci-G₁ Orange encodes *CoralHue*[®] monomeric Kusabira-Orange2 (mKO2) fused to a part of human Cdt1 (hCdt1: Cdc10 dependent transcript 1). It is possible to use PM051 for Fucci transgenic strain, B6.Cg-Tg(Fucci)596Bsi mice which express Fucci-G₁ Orange.



Immunohistochemical detection of mKO2 on frozen section of B6.Cg-Tg (Fucci) 596Bsi mouse embryonic brain (E12) with PM051 (1) and Fucci-G₁ Orange own fluorescence (2).

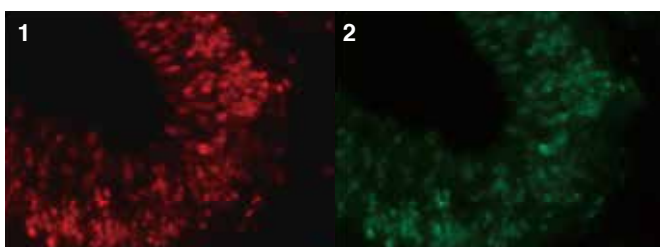


Immunocytochemical detection of mKO2 in Fucci-G₁ Orange transfected HeLa cells with PM051.

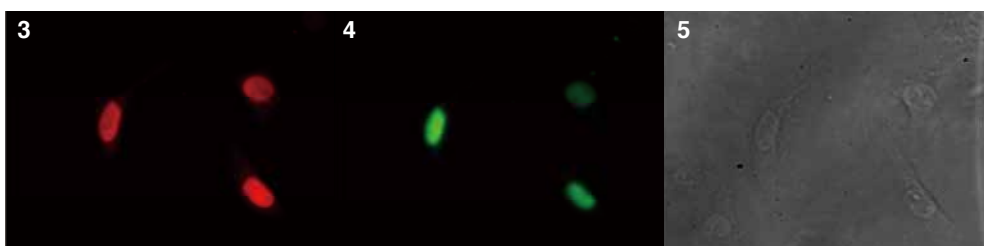
3: Anti-mKO2
4: Fucci-G₁Orange
5: Transmission light

Anti-monomeric Azami-Green 1 (polyclonal antibody): code No. PM052

PM052 is available for immunostaining of “Fucci-S/G₂/M Green”. Fucci-S/G₂/M Green encodes *CoralHue*[®] humanized monomeric Azami-Green1 (hmAG1) fused to a part of human Geminin (hGeminin). It is possible to use PM052 for Fucci transgenic strain, B6.Cg-Tg(Fucci)504Bsi mice which express Fucci-S/G₂/M Green.



Immunohistochemical detection of mAG1 on frozen section of B6.Cg-Tg (Fucci) 504Bsi mouse embryonic brain (E13) with PM052 (1) and Fucci-S/G₂/M Green own fluorescence (2).

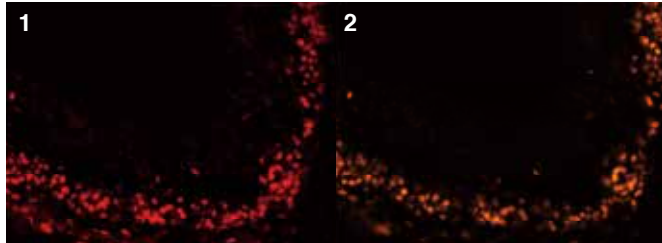


Immunocytochemical detection of mAG1 in Fucci-S/G₂/M Green transfected HeLa cells with PM052.

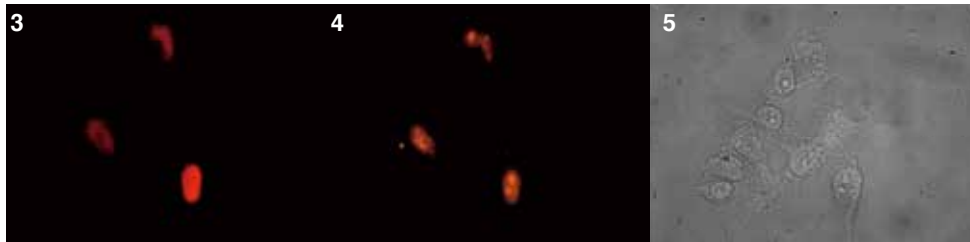
3: Anti-mAG1
4: Fucci-S/G₂/M Green
5: Transmission light

Anti-monomeric Kusabira-Orange 2 (monoclonal antibody): Code No. M168-3

M168-3 is available for immunostaining of "Fucci-G₁ Orange". It is possible to use M168-3 for Fucci transgenic strain, B6.Cg-Tg(Fucci)596Bsi mice which express Fucci-G₁ Orange.



Immunohistochemical detection of mKO2 on frozen section of B6. Cg-Tg (Fucci) 596Bsi mouse embryonic brain (E12) with M168-3 (1) and Fucci-G₁ Orange own fluorescence (2).



Immunocytochemical detection of mKO2 in Fucci-G₁ Orange transfected HeLa cells with M168-3.

3: Anti-mKO2
4: Fucci-G₁ Orange
5: Transmission light

CoralHue® Antibody Product List

Code No.	Product	Clone	Isotype	Size	Applications
M102-3	anti-Azami-Green	2F11	mouse IgG1 κ	100 μ g	WB
M103-3	anti-Azami-Green	3D10	mouse IgG2a κ	100 μ g	IP
PM011	anti-Azami-Green	Polyclonal	rabbit IgG	100 μ L	WB
M117-3	anti-Dronpa-Green	4D12	mouse IgG2a	100 μ g	WB
M118-3	anti-Dronpa-Green	2F6	mouse IgG2b	100 μ g	IP
M106-3	anti-Kaede	2F4	mouse IgG1 κ	100 μ g	IP
M125-3	anti-Kaede	3B1	mouse IgG1	100 μ g	WB
PM012	anti-Kaede	Polyclonal	rabbit IgG	100 μ L	WB
M126-3	anti-Keima-Red	2F7	mouse IgG2a	100 μ g	WB
M127-3	anti-Keima-Red	3C9	mouse IgG1	100 μ g	IP
M128-3	anti-Kikume Green-Red	5B3	mouse IgG2b	100 μ g	WB
M129-3	anti-Kikume Green-Red	2D3	mouse IgG2b	100 μ g	IP
M104-3	anti-Kusabira-Orange	1H7	mouse IgG1 κ	100 μ g	WB
M105-3	anti-Kusabira-Orange	2G9	mouse IgG1 κ	100 μ g	IP
M116-3	anti-Midoriishi-Cyan	2C1	mouse IgG2b	100 μ g	IP
M130-3	anti-Midoriishi-Cyan	5B7	mouse IgG1	100 μ g	WB
PM052	anti-monomeric Azami-Green 1	Polyclonal	Rabbit Ig(aff.)	100 μ L	WB, IP, IC, IH
M149-3	anti-monomeric Kusabira-Green C-terminal fragment	21B10	mouse IgG2a	100 μ g	WB
M148-3	anti-monomeric Kusabira-Green N-terminal fragment	1E6	mouse IgG2b	100 μ g	WB
M168-3	anti-monomeric Kusabira-Orange 2	3B3	mouse IgG1 κ	100 μ g	WB, IP, IC, IH
PM051	anti-monomeric Kusabira-Orange 2	Polyclonal	Rabbit Ig(aff.)	100 μ L	WB, IP, IC, IH

(aff.): affinity purified

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

Product List

CoralHue® Fluorescent protein vectors

Fluorescent protein	Abbr.	Form	Excitation maxima (nm)	Emission maxima (nm)	S1	MC1	MN1	MCLinker	MNLinker
Midoriishi-Cyan	MiCy1	Dimer	472	495	AM-V0061				
	mMiCy1	Monomer	470	496	AM-V0111				
	hmMiCy1	Monomer	470	496		AM-V0115	AM-V0116	AM-V0119	AM-V0110
Umikinko-Green	mUKG1	Monomer	483	499	AM-V0161				
	hmUKG1	Monomer	483	499	AM-V0164	AM-V0165	AM-V0166		
Azami-Green	AG	Tetramer	492	505	AM-V0021				
	mAG1	Monomer	492	505	AM-V0031	AM-V0032	AM-V0033		
	hmAG1	Monomer	492	505	AM-V0034	AM-V0035	AM-V0036	AM-V0039	AM-V0030
Kusabira-Orange	KO1	Dimer	548	561	AM-V0041				
	mKO1	Monomer	548	559	AM-V0051	AM-V0052	AM-V0053		
	mKO2	Monomer	551	565	AM-V0141				
	hKO1	Dimer	548	561	AM-V0044	AM-V0045	AM-V0046		
	hmKO1	Monomer	548	559	AM-V0054	AM-V0055	AM-V0056	AM-V0059	AM-V0050
	hmKO2	Monomer	551	565		AM-V0145	AM-V0146	AM-V0149	AM-V0140
Keima-Red	dKeima570	Dimer	440	570	AM-V0121				
	hdKeima570	Dimer	440	570	AM-V0124			AM-V0129	AM-V0120
	dKeima-Red	Dimer	440	616	AM-V0101				
	mKeima-Red	Monomer	440	620	AM-V0091		AM-V0093		
	hdKeima-Red	Dimer	440	616	AM-V0104			AM-V0109	AM-V0100
	hmKeima-Red	Monomer	440	620	AM-V0094			AM-V0099	AM-V0090
Dronpa Green	DG1	Monomer	503	518	AM-V0071	AM-V0072	AM-V0073		
	hDG1	Monomer	503	518				AM-V0079	AM-V0070
	DG3	Monomer	491	514	AM-V0131				
Kaede	Kaede	Tetramer	508/572	518/580	AM-V0011	AM-V0012	AM-V0013		
Kikume Green-Red	KikGR1	Tetramer	507/583	517/593	AM-V0081	AM-V0082	AM-V0083		
	mKikGR1	Monomer	505/580	517/591	AM-V0151				
	hKikGR1	Tetramer	507/583	517/593	AM-V0084	AM-V0085	AM-V0086	AM-V0089	AM-V0080
	hmKikGR1	Monomer	505/580	517/591				AM-V0159	AM-V0150

S1: Vectors for subcloning MC1, MN1: Expression vectors MCLinker, MNLinker: Expression vectors added flexible linkers to h: Humanized-codon

Organelle targeting vectors

Fluorescent protein	Abbr.	Form	Excitation maxima (nm)	Emission maxima (nm)	Mitochondria	Endoplasmic reticulum	Plasma membrane	Nucleoplasm
Azami-Green	AG	Tetramer	492	505				AM-V0214
	mAG1	Monomer	492	505	AM-V0201	AM-V0202	AM-V0203	
Kusabira-Orange	KO1	Dimer	548	561				AM-V0234
	mKO1	Monomer	548	559	AM-V0221	AM-V0222	AM-V0223	
Keima-Red	mKeima-Red	Monomer	440	620	AM-V0251		AM-V0253	
	hdKeima-Red	Dimer	440	616				AM-V0274

Product List

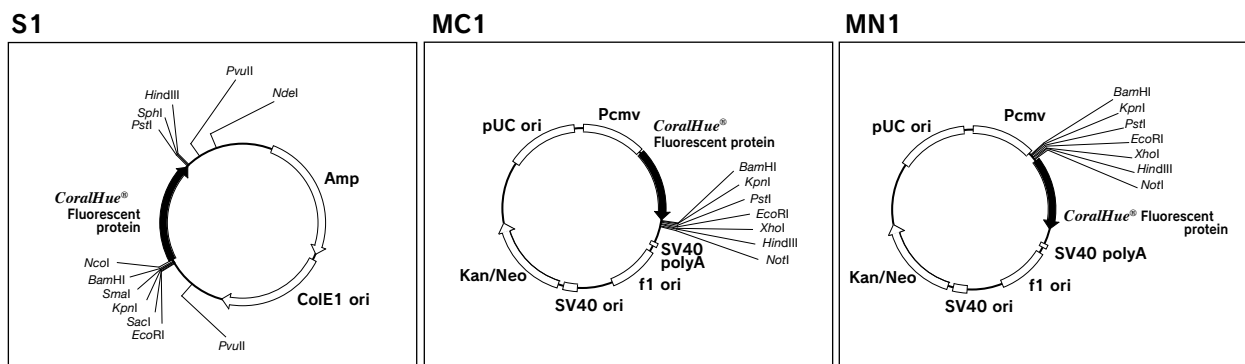
Fucci (Fluorescent Ubiquitination-based Cell Cycle Indicator)

Code No.	Product	Size
AM-V9001	pFucci-G ₁ Orange (cloning vector)	20 µg
AM-V9003	pFucci-G ₁ Orange (expression vector)	20 µg
AM-V9014	pFucci-S/G ₂ /M Green (cloning vector)	20 µg
AM-V9016	pFucci-S/G ₂ /M Green (expression vector)	20 µg
AM-V9034	pFucci-S/G ₂ /M Green(N+C) (cloning vector)	20 µg
AM-VS0601	Fucci Set (AM-V9001 + AM-V9014)	20 µg + 20 µg
AM-VS0602	Fucci Set (AM-V9003 + AM-V9016)	20 µg + 20 µg
AM-VS0603	Fucci Set (AM-V9001 + AM-V9016)	20 µg + 20 µg
AM-VS0604	Fucci Set (AM-V9003 + AM-V9014)	20 µg + 20 µg
AM-VS0605	Fucci Set (AM-V9001 + AM-V9034)	20 µg + 20 µg
AM-VS0606	Fucci Set (AM-V9003 + AM-V9034)	20 µg + 20 µg

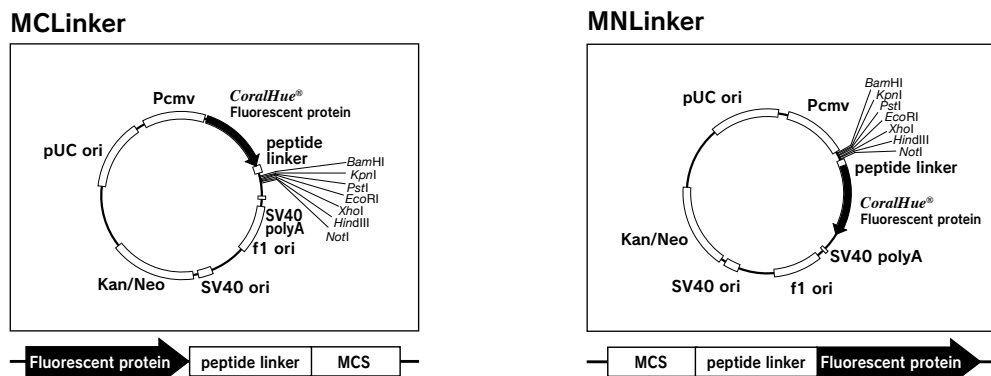
Kits

Code No.	Product	Size
4690	APOPCYTO™ Annexine V-Azami-Green	1 system
AM-1001	AMAP™ Multi Site-directed Mutagenesis Kit	20 tests
AM-1100	CoralHue® Fluo-chase Kit	1 system

Fluorescent protein vector map



Fluorescent protein vector map with linkers



References

Midoriishi-Cyan

- 1) Karasawa S. et al., Biochem. J. 381, 307-312 (2004)

Umikinoko-Green

- 1) Tsutsui H. et al., Nat. Methods 5, 683-685

Azami-Green

- 1) Karasawa S. et al., J. Biol. Chem. 278, 34167-34171 (2003)
- 2) Shaner N.C. et al., Nat. Methods 2, 905-909 (2005)
- 3) Gruber D.F. et al., Biol. Bull. 215, 143-154 (2008)
- 4) Ebisawa T. et al., Acta. Crystallogr. Sect. F. Struct. Biol. Cryst. Commun. 65, 1292-1295 (2009).
- 5) Kiss C. et al., Protein Eng. Des. 22, 313-323 (2009)

Kusabira-Orange

- 1) Karasawa S. et al., Biochem. J. 381, 307-312 (2004)
- 2) Shaner N.C. et al., Nat. Methods 2, 905-909 (2005)
- 3) Niwa H. et al., Cell 123, 917-929 (2005)
- 4) Goedhart J. et al., PLoS ONE 2, e1011 (2007)
- 5) Livet J. et al., Nature 450, 56-62 (2007)
- 6) Matsunari H. et al., Cloning and Stem Cells 10, 313-324 (2008)
- 7) Sanuki S. et al., J. Gene Med. 10, 965-971 (2008)

Keima

- 1) Kogure T. et al., Nat. Biotechnol. 24, 577-581 (2006)
- 2) Kawano H. et al., Nat. Methods 5, 373-374 (2008)
- 3) Kogure T. et al., Methods 45, 223-226 (2008)
- 4) Matsumoto Y. et al., J. Gene Med. 11, 615-623 (2009)
- 5) Violot S. et al., J. Am. Chem. Soc. 131, 10356-10357 (2009)
- 6) Henderson J.N. et al., J. Am. Chem. Soc. 131, 13212-13213 (2009)

Dronpa-Green

- 1) Ando R. et al., Science 306, 1370-1373 (2004)
- 2) Kurokawa K. et al., Mol. Biol. Cell 16, 4294-4303 (2005)
- 3) Habuchi S. et al., Proc. Natl. Acad. Sci. U.S.A. 102, 9511-9516 (2005)
- 3) Habuchi S. et al., Photochem. Photobiol. Sci. 5, 567-576 (2006).
- 4) Dedeker P. et al., Biophys. J. 91 L45-47 (2006)
- 5) Ando R. et al., Biophys. J. 92, L97-99 (2007)
- 6) Fron E. et al., J. Am. Chem. Soc. 129, 4870-4871 (2007)
- 7) Mizuno H. et al., Photochem. Photobiol. Sci. 9, 239-248 (2010)

Kaede

- 1) Ando R. et al., Proc. Natl. Acad. Sci. U.S.A. 99, 12651-12656 (2002)
- 2) Stephens D. Trends in Cell Biol. 12 550 (2002)
- 3) Mizuno H. et al., Mol. Cell 12, 1051-1058 (2003)
- 4) Arimura S. et al., Proc. Natl. Acad. Sci. U.S.A. 101, 7805-7808 (2004)
- 5) Dittich P.S. et al., Biochys. J. 89, 3446-3455 (2005)
- 6) Sato T. et al., Genesis 44, 136-142 (2006)
- 7) Mutoh T. et al., Exp. Neurol. 200, 430-437 (2006)
- 8) Raab- Graham K.F. et al., Science 314, 144-148 (2006)
- 9) Hosoi H. et al., J. Phys. Chem. B. 110, 22853-22860 (2006)
- 10) Hatta K. et al., Nat. Protoc. 1, 960-967 (2006)
- 11) Pisharath H. et al., Mech. Dev. 124, 218-229 (2007)
- 12) Davidson J.M. et al., Dev. Biol. 304, 811-824 (2007)
- 13) Scott E.K. et al., Nat. Methods 4, 323-326 (2007)
- 14) Stark D.A and Kulesa P.M. Dev. Dyn. 236, 1483-1594 (2007)
- 15) Watanabe W. et al., Opt. Express 15, 2490-2498 (2007)
- 16) Leung K.M. and Holt C.E. Nat. Protoc. 3, 1318-1327 (2008)
- 17) Tomura M. et al., Proc. Natl. Acad. Sci. U.S.A. 105, 10871-10876 (2008)
- 18) Schmidt A. et al., Traffic 10, 2-15 (2009)
- 19) Tomura M. et al., J. Clin. Invest. 120, 883-893 (2010)
- 20) Tomura M. et al., J. Immunol. 184, 4646-4653 (2010)

Kikume Green-Red

- 1) Tsutsui H. et al., EMBO Rep. 6, 233-238 (2005)
- 2) Hatta K. et al., Nat. Protoc. 1, 960-967 (2006)
- 3) Stark D.A. et al., Dev. Dyn. 236, 1583-1594 (2007)
- 4) Habuchi S. et al., PLoS ONE 3, e3944 (2008)
- 5) Tsutsui H. et al., Chem. Biol. 16, 1140-1147 (2009)
- 6) Mizuno H. Photochem. Photobiol. Sci. 9, 239-248 (2010)

Fluo-Chase Kit

- 1) Ueyama T. et al., J. Immunol. 181, 629-640 (2008)
- 2) Hamatake M. et al., Cancer Sci. 100, 95-102 (2009)
- 3) Hashimoto J. et al., J. Biomol. Screen. 14, 970-979 (2009)
- 4) Yoshida T. et al., Microbiol. Immunol. 53, 629-635 (2009)

Fucci

- 1) Sakaue-Sawano A. et al., Cell 132, 487-498 (2008)
- 2) Sakaue-Sawano A. et al., Chem. Biol. 15 1243-1248 (2008)
- 3) Ishikawa M. et al., Biochem. Biophys. Res. Commun. 389, 426-430 (2009)

Visit our website for more information.

<http://www.amalgaam.co.jp>

[https:// ruo.mbl.co.jp](https://ruo.mbl.co.jp)

Data sheets, vector sequences, spectral graphs,...

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

CoralHue® fluorescent proteins were co-developed by Amalgaam and the Laboratory for Cell Function and Dynamics, the Advanced Technology Development Center, the Brain Science Institute, and RIKEN.

The use of **CoralHue®** fluorescent proteins requires a license agreement from Amalgaam. Amalgaam grants non-profit research organizations a royalty-free international non-exclusive limited license to use this product for non-commercial research use. This license excludes the right to sell or transfer the product, its components, or modifications of the product to third parties. Any other use requires a license agreement.

The use of this product by for-profit organizations, for either commercial or non-commercial use, requires a license agreement from Amalgaam. For additional licensing information, please contact us at 'support@mbi.co.jp'.