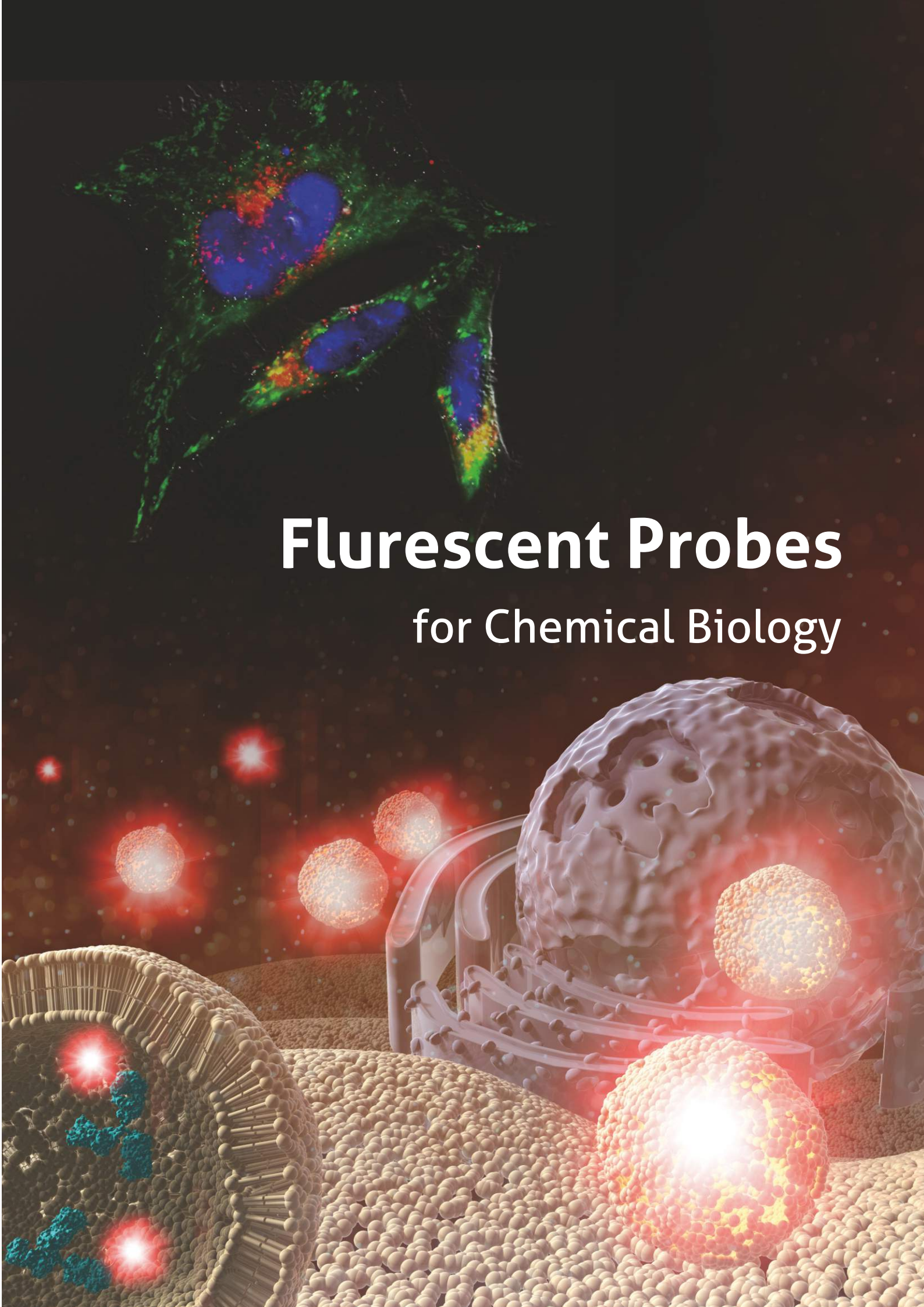


Flurescent Probes

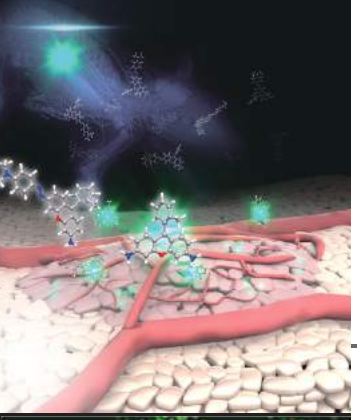
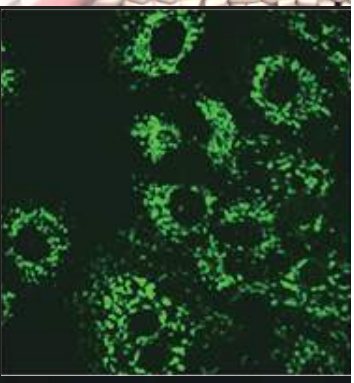
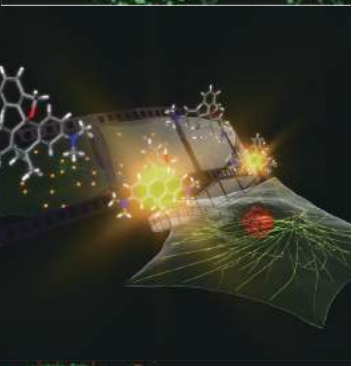
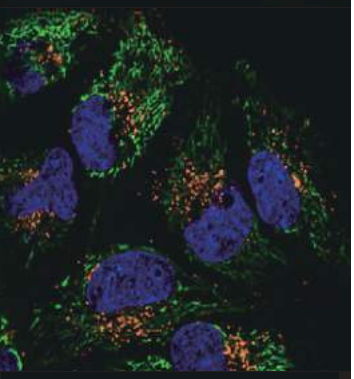
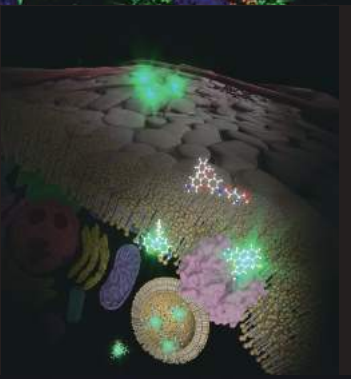
for Chemical Biology

Creating New Fluorescent Probes for Chemical Biology

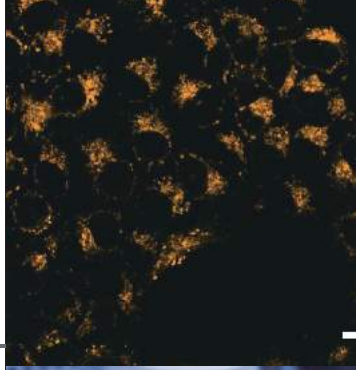
GORYO Chemical creates innovative fluorescent probes through licensing and in-house value addition by expanding applications in cell biology and expedi-ently bringing them to researchers in academia, small biotech as well as large pharmaceutical companies.

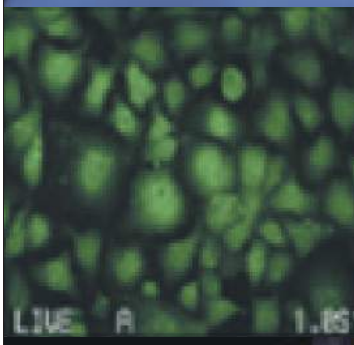
Unique partnerships with life science instrument companies both in the invitro and invivo.

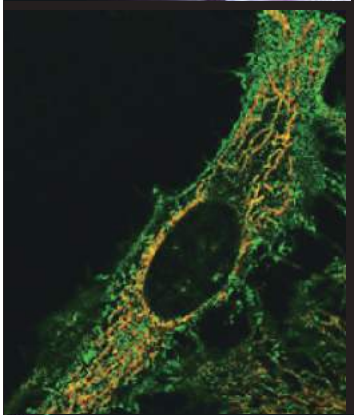
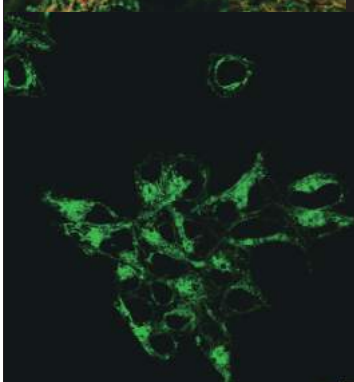
Experimentation stages create solutions to understand both hypothesis driven as well as discovery research. A solid background in chemistry pertain-ing to optical probes that are uniquely tailored to elucidate cellular mecha-nisms in biology through relationships with Tokyo University and Hokkaido University (Nobel prize winner Prof. Suzuki) makes Goryo uniquely positioned to be a premier supplier of fluorescent reagents bridging the invitro to invivo, and to human translation.


Contents





NOFluor™ Series

P.27

Diaminofluorescein-2 (DAF-2) ／Diaminofluorescein-2 diacetate (DAF-2 DA)
Diaminofluorescein-FM (DAF-FM) ／Diaminofluorescein-FM diacetate (DAF-FM DA)
Diaminorhodamine-4M (DAR-4M) ／Diaminorhodamine-4M acetoxymethyl ester (DAR-4M AM)

ROSFluor™ Series

P.30

NiSPY-3 (Nitrative Stress Sensing Pyrromethene Dye)

Hydroxyphenyl Fluorescein (HPF) / Aminophenyl Fluorescein (APF)
Measurement of reactive oxgen species

NIRFluor™ Series

P.34

ICG Near Infrared Fluorescent Dyes For in vivo imaging using near infrared fluorescent dyes

ICG Line-up

P.36

Line-up of ICG near infrared fluorescent dyes

STELLA Fluor™ Series

P.38

A series of fluorescent dye originally released from Goryo Chemical Inc.

Bioluminescent Series

P.39

POLARIC™ Series

P.40

POLARIC™-500c6F Staining both mitochondria and cell membrane by a single dye

Labeling Services

P.41

POLARIC™ Labeling services Custom Services ／ Other Fluorophores Labeling services Custom Services

Contract Uptake Assay Systems of Fluorescent-Labeled Substances Order Made Services

For the study of cancer or hypoxia

Hypoxia detection probe

MAR

Easily available for live-cell imaging

As sensitive as pimonidazole

Available for flow cytometry

Available for the detection of hypoxia in tissue

Feature 1.

Easily available for live-cell imaging

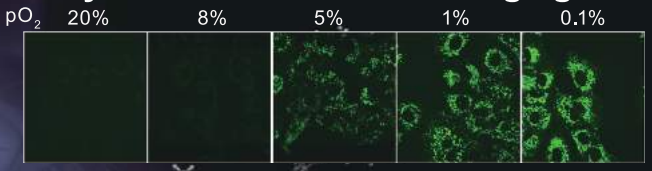
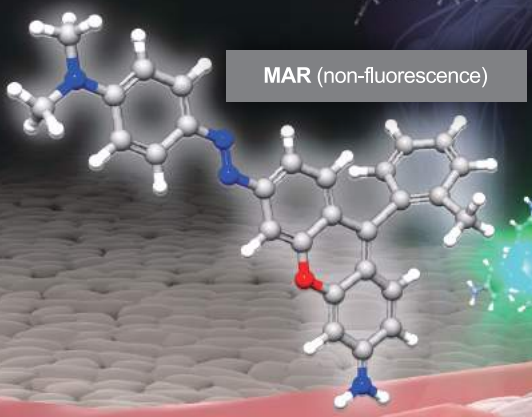
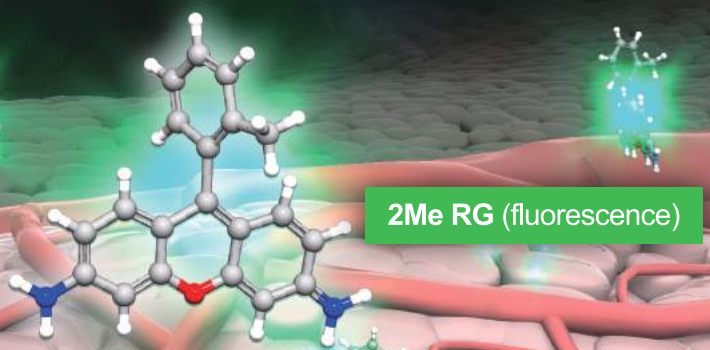


Fig. 1. Fluorescent imaging of A549 cell under the various concentration of oxygen.
Fluorescent intensity increased as content of oxygen decreased. Pimonidazole is used to be generally used for the detection of hypoxia, but cell fixation and immunostaining are necessary. However, fluorescent live-cell imaging of hypoxia is available only by addition of MAR to the living cells.

Fig. 2. (Background image) Reduced reaction of MAR under hypoxia
Though MAR is non-fluorescent, reductive cleavage in the azo base of the probe occurs by the reductive enzyme under hypoxia, and 2Me RG is generated which makes bright green fluorescence.



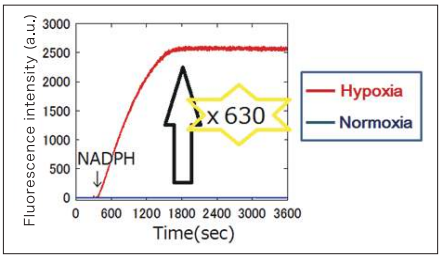


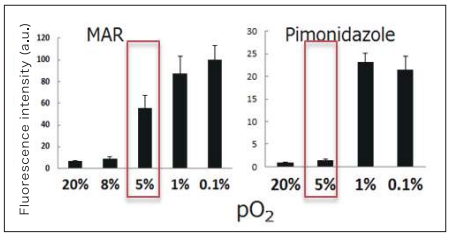
Feature1

Easily available for live-cell imaging

Fig. 3. Response to hypoxia in vitro.

50 μM NADPH was introduced to 5 μM MAR in the existence of rat liver microsomes, under hypoxia or normoxia. MAR was reduced only under hypoxia to yield 100 times brighter fluorescence.





As sensitive as pimonidazole

Feature2

Fig. 4. Fluorescent intensity in A549 cell under hypoxia, stained by MAR or pimonidazole.

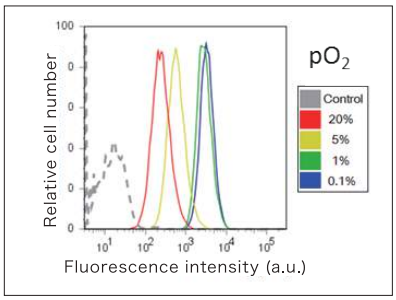
A549 cells were observed after staining by MAR or pimonidazole under the various concentrations of oxygen. While pimonidazole responded to the concentration of oxygen under 1%, MAR responded to the oxygen concentration of about 5%.

Feature3

Available for flow cytometry

Fig. 5. Analysis by flow cytometry of A549 cells stained by MAR under hypoxia.

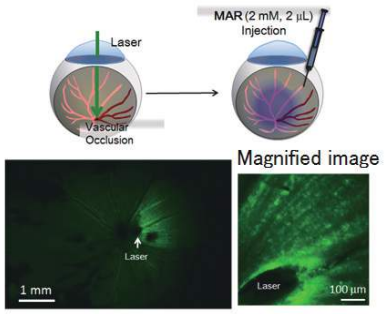
A549 cells were analyzed by flow cytometry after the incubation for 6 hours under the various oxygen concentrations and stained by 1 mM MAR. Fluorescent intensity increased as the oxygen concentration decreased, indicating that the probe is available for flow cytometry.



Application example

Available for the detection of hypoxia in tissue

Fig. 6. Imaging of retinal artery occlusion in rat.
Retinal artery occlusion was created in rat retina by laser irradiation and the fluorescent image was captured. Increase in the fluorescent intensity by MAR was observed specifically in the part of the retina in which occlusion and hypoxia had occurred. (Data were kindly offered by Prof. Toru Nakazawa, Dr. Yuji Tanaka and Dr. Satoshi Tsuda).



Code No.	Product	Outline	Size
A101-01	MAR	Hypoxia detection probe	25 μg×5

Reference

1. W. Piao, S. Tsuda, Y. Tanaka, S. Maeda, F. Liu, S. Takahashi, Y. Kushida, T. Komatsu, T. Ueno, T. Terai, T. Nakazawa, M. Uchiyama, K. Morokuma, T. Nagano, K. Hanaoka Angew Chem Int Ed 52 (49) 13028–13032, (2013)

Novel imaging probe overcoming the problems of dSTORM

Fluorescent probe for super-resolution live-cell imaging

HMSiR series

- **Usable for super-resolution live-cell imaging under the physiological condition**
- **The fluorescent probe shows blinking spontaneously without high-power laser irradiation**

dSTORM (direct stochastic optical reconstruction microscopy) is one of the super-resolution technique utilizing the blinking of the fluorescent probes. The newly released HMSiR displays spontaneously blinking in light emission. By using this probe, we can operate super-resolution imaging without thiols, oxygen scavengers or irradiation of high-power laser, all of which were necessary for causing the blinking of fluorescent dyes in the previous dSTORM observation. Therefore, this probe enables live-cell and super-resolution imaging with low-power excitation light under the physiological condition.

Available for the super-resolution live-cell imaging under the physiological condition without thiols or oxygen scavengers

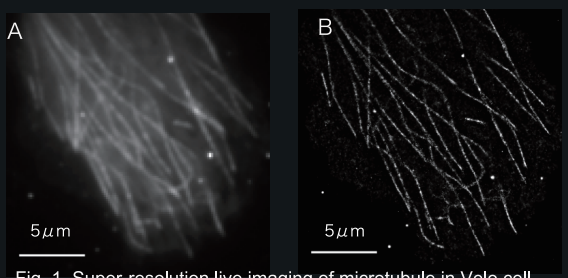


Fig. 1. Super-resolution live imaging of microtubule in Velo cell. Velo cells, in which HaloTag®-β-tubulin was expressed, were stained by HMSiR-Halo and observed by TIRF microscopy. A: averaged image. B: super-resolution image. Super-resolution imaging with HMSiR did not need thiols or oxygen scavengers and was operated under the physiological condition.

Observation without high-power laser irradiation minimizes the damage of the cell

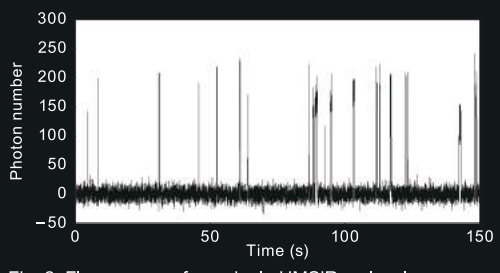


Fig. 2. Fluorescence from single HMSiR molecule. HMSiR displays spontaneously blinking without irradiation of high-power laser (~1 kW cm−2) which used to be necessary for the dSTORM observation (laser power: 100 W cm−2, correspondence to On/Off state). It allows us to minimize the damage of the cell by the laser irradiation used in the previous dSTORM observation.

Reference:
Uno S, Kamiya M, Yoshihara T, Sugawara K, Okabe K, Tarhan MC, Fujita H, Funatsu T, Okada Y, Tobita S, Urano Y. A spontaneously blinking fluorophore based on intramolecular spirocyclization for live-cell super-resolution imaging. **Nature Chem.** 6, 681-689, 2014.

Line-up of the probes for super-resolution imaging

	Code.No.	Product Name	Size	Remarks
For live cells	A201-01	HMSiR-Halo	15nmol	Affinity ligands to HaloTag® fusion proteins (In Preparation)
	A201-02	HMSiR-Halo	30nmol	
For fixed cells	A202-01	HMSiR labeled Goat anti-mouse IgG (whole)	100ug	IgG antibody, from goat, labeled by HMSiR
	A203-01	HMSiR labeled Goat anti-rat IgG (whole)	100ug	
	A204-01	HMSiR labeled Goat anti-rabbit IgG (whole)	100ug	
	A205-01	HMSiR labeled Goat anti-mouse IgG F(ab)'	100ug	F(ab)'fragment of IgG antibody labeled by HMSiR. For higher resolution imaging than that with whole IgG antibodies.
	A206-01	HMSiR labeled Goat anti-rat IgG F(ab)'	100ug	
	A207-01	HMSiR labeled Goat anti-rabbit IgG F(ab)'	100ug	
	A208-01	HMSiR-NHS	100ug	Labeling proteins or antibodies by amide bond.
	A209-01	HMSiR-Maleimide	100ug	Labeling thiols of proteins or antibodies with maleimide.

Reference

1. Uno SN, Kamiya M, Yoshihara T, Sugawara K, Okabe K, Tarhan MC, Fujita H, Funatsu T, Okada Y, Tobita S, Urano Y. Nat Chem. 2014 681-689.

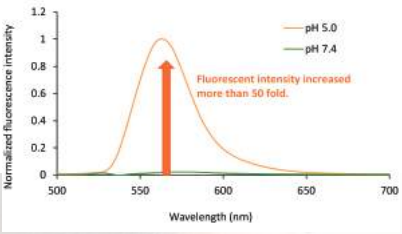
Brightest pH sensitive fluorescent probe.
Bright even after labeling protein.

AcidiFluor™ Series

AcidiFluor™ ORANGE is fluorescent probe that increase greatly its fluorescence under the acidic condition. Acidic organelles, such as lysosomes, late endosomes and secretory granules, are stained by the probe.
AcidiFluor™ ORANGE-NHS is usable for labeling amino acids of proteins or nucleic acids.
It forms stable covalent bond only by mixing with antibodies or proteins that have amino acids.

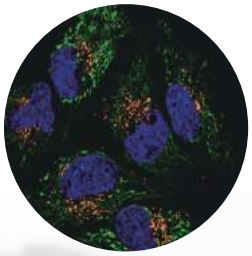
- **For the study of endocytosis or phagocytosis**
- **The orange fluorescence is applicable to multi-color imaging**
- **Highly resistant to bleaching**

AcidiFluor™ ORANGE



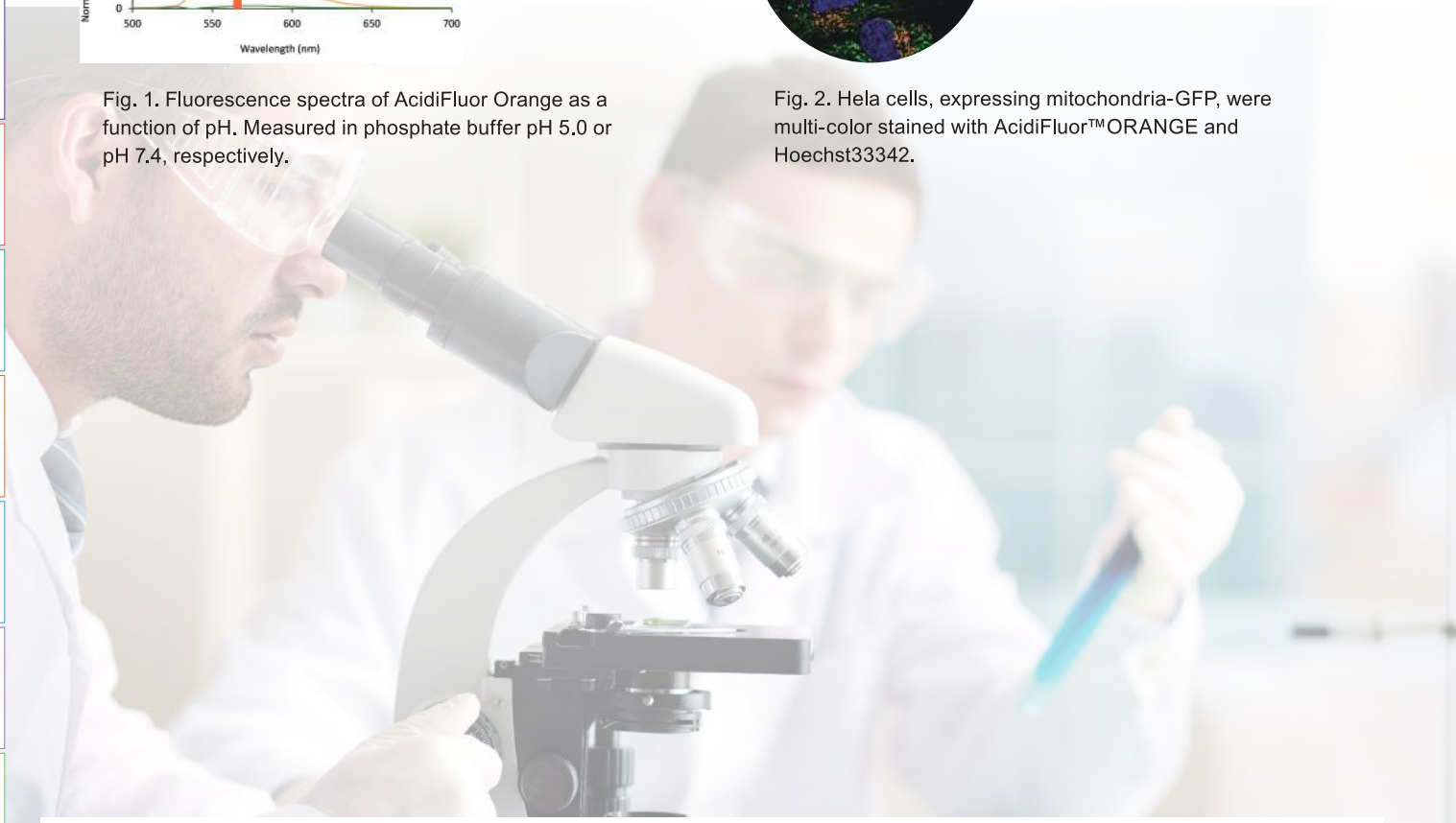
Fluorescent intensity of AcidiFluor Orange at pH 5.0 increased 50 fold to that in the pH 7.4 buffer.

Fig. 1. Fluorescence spectra of AcidiFluor Orange as a function of pH. Measured in phosphate buffer pH 5.0 or pH 7.4, respectively.



AcidiFluor™ ORANGE is usable for live-cell multi-color imaging with blue color of Hoechst33342 in nucleus, green color of GFP in mitochondria, and orange color in lysosomes.

Fig. 2. Hela cells, expressing mitochondria-GFP, were multi-color stained with AcidiFluor™ORANGE and Hoechst33342.

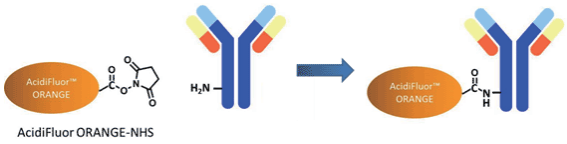


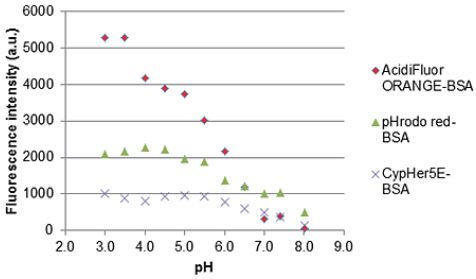
YouTube GoryoChemical http://www.youtube.com/watch?v=Vdprn0MmPRE

On our website, many other movies, such as phagocytosis of ZymosanA labeled with AcidiFluor™ORANGE-NHS by RAW264.7 cell, are available.

AcidiFluor™ ORANGE-NHS

AcidiFluor™ ORANGE-NHS is able to detect acidic condition with high sensitivity even after conjugation with proteins. The fluorescence intensity increases 10 times greater in pH 5.0, which corresponds to the environment in acidic organelles, than in physiological pH 7.4. It also shows high resistance to photo bleaching.





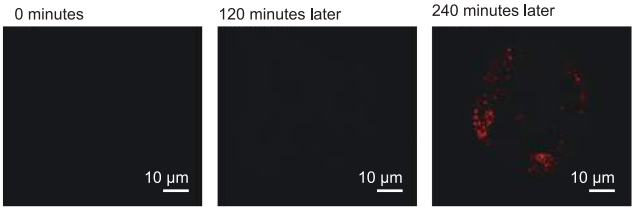
GC304 AcidiFluor™ORANGE Labeling Kit

Fig.3. Fluorescent intensities of each pH probes conjugated with BSA.
(AcidiFluor™ ORANGE-NHS: λex 520, λem 565, pHrodo™-NHS (Life Technology Corporation): λex 545, λem 580, CypHer™5E-NHS (GE Healthcare) : λex 625, λem 665) in various pH conditions (from pH 3.0 to 8.0). Fluorescent intensity of AcidiFluor™ ORANGE-NHS increased sensitively in acidic conditions, however, changes in fluorescent intensity by the pH concitions were only small in pHrodo and CypHer5E.

Application example　AcidiFluor™ ORANGE-NHS

Fig. 4. Endocytosis of anti-EGFR antibody labelled with AcidiFluor™ ORANGE-NHS by A431 cell.

Anti-EGFR antibody labelled with AcidiFluor™ ORANGE-NHS was taken into A431 cell. Fluorescent intensity was increased specifically in lysosomes in which pH was low.



Code No.	Product	Size	Remarks
GC301	AcidiFluor™ ORANGE	10μg × 20	For live cell imaging of lysosome.
GC3011	AcidiFluor™ ORANGE	10μg × 10	For live cell imaging of lysosome. (Small size for trial)
GC302	AcidiFluor™ ORANGE-NHS	1mg	For labeling antibodies or proteins. Just mixing.
GC303	AcidiFluor™ ORANGE-NHS	For 5 times	Subdivided packaging. For labeling antibodies or proteins.
GC304	AcidiFluor™ ORANGE Labeling Kit	For 5 times	All-in-one kit for labeling.
GC305	AcidiFluor™ ORANGE-Zymosan A	1mg	For the study of phagocytosis.
GC306	AcidiFluor™ ORANGE-Dextran 10k	1mg	For the study of phagocytosis.
GC307	AcidiFluor™ ORANGE-Beads 500	500nm/250μg	Nano sized silica particles were labeled with AcidiFluor™ ORANGE. For the study of cellular uptake such as endocytosis.
GC3071	AcidiFluor™ ORANGE-Beads 1000	1μm/250μg	Nano sized silica particles were labeled with AcidiFluor™ ORANGE. For the study of cellular uptake such as endocytosis.
GC308	AcidiFluor™ ORANGE-wBeads 500	500nm/250μg	Beads sensors for pH, involving FITC inside. Easy to be tracked.
GC3081	AcidiFluor™ ORANGE-wBeads 1000	1μm/250μg	Beads sensors for pH, involving FITC inside. Easy to be tracked.
GC309	AcidiFluor™ ORANGE-Transferin	1mg	For the study of endocytosis.

References

1. Asanuma D, Takaoka Y, Namiki S, Takikawa K, Kamiya M, Nagano T, Urano Y, Hirose K: Acidic-pH-activatable Fluorescence Probes for Visualizing Exocytosis Dynamics. Angew Chem Int Ed, 2014, doi: 10.1002/anie.201402030
2. Masayuki Isa, Daisuke Asanuma, Shigeyuki Namiki, Kazuo Kumagai, Hirotatsu Kojima, Takayoshi Okabe, Tetsuo Nagano, and Kenzo Hirose, ACS Chem. Biol., 2014, 9 (10), pp 2237–2241, "High-Throughput Screening System To Identify Small Molecules That Induce Internalization and Degradation of HER2"
3. Watanabe R, Soga N, Fujita D, Tabata KV, Yamauchi L, Hyeon Kim S, Asanuma D, Kamiya M, Urano Y, Suga H, Noji H. Nat Commun. 2014 Jul 24;5:4519. doi: 10.1038/ncomms5519., "Arrayed lipid bilayer chambers allow single-molecule analysis of membrane transporter activity"

Fluorescent probes with red or near infra-red fluorescence for detecting calcium ion.
Usable together with GFP.

CalFluor™ Series

- **Fluorescence in red (609 nm) or in near infra-red region (664 nm).**
- **Sensitively detecting calcium ion**
- **Usable for multicolor imaging**

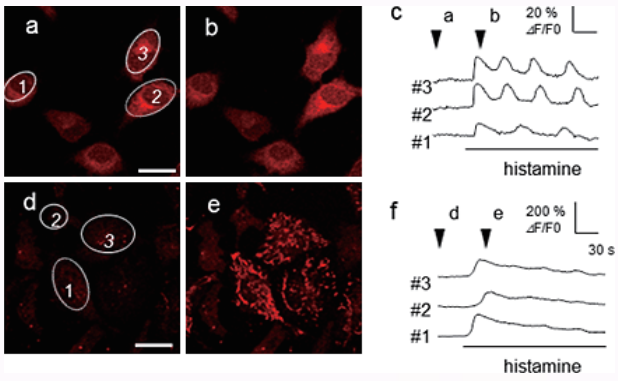
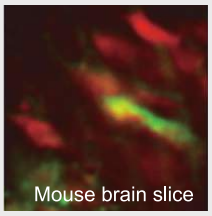
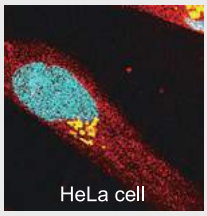


Fig. 1. (a-c) Fluorescent images of HeLa cells after injection of CaTM-2™AM and histamine stimulation (a, b). Change in the fluorescent intensities in the regions indicated in panel a (c).

CaTM-2™ distributed in the cytoplasm and detected the change in the concentration of calcium ion. (d-f) Similar experiments with Rhod-2, conventional red-color probe for calcium ion, as (a-c). Since Rhod-2 localized in mitochondria, it could only detect change in the calcium concentration inside mitochondria.



Near infra-red fluorescence
by CaSiR™-1/ CaSiR-1™ AM



Red fluorescence
by CaTM-2™/ CaTM-2™ AM

CaSiR-1™/ CaSiR-1™ AM

Fluorescent probes with near infra-red fluorescence for detecting calcium ion.

CaSiR-1™/CaSiR-1™ AM are fluorescent probes with near infra-red fluorescence, at maximum fluorescence at 664 nm, for detecting calcium ion. They are usable for multi-color imaging with fluorescent proteins and other fluorescent dyes, which have fluorescence in visible region, such as YFP and RFP. The fluorescence in near infra-red region have advantage of optical transparency in living tissue and of low background noise, and causes little damage to cells or tissues.


CaSiR-1™ AM Kit

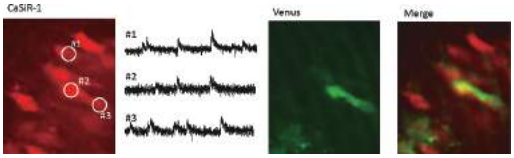


Fig. 2. Ca^{2+} imaging in mouse brain neurons.

Ca^{2+} imaging was performed after CaSiR-1™AM was applied on the brain slice of mouse in which Venus, a valiant of YFP, was expressed. Red: CaSiR-1™, Green: Venus. Change in the fluorescent intensity showed the temporal increase in Ca^{2+} ion in cells accompanying stimulation in brain neuron. This study was performed to assess the difference of Ca^{2+} in the neurons expressing YFP and in the neuron in which YFP was not expressed. Ca^{2+} imaging or various multi-color imaging of cells labeled by fluorescent proteins is possible like this.

CaTM-2™/ CaTM-2™ AM

Fluorescent probes with red fluorescence for detecting calcium ion.

This is a calcium probe with red fluorescence, ideal for the analysis of the calcium ion in cytoplasm. It detects fluorescence sensitively corresponding to the concentration of calcium ion. CaTM™-2/CaTM-2™ AM are fluorescent probes with red fluorescence, at maximum fluorescence at 609 nm, for detecting calcium ion. They are usable for multi-color imaging with fluorescent proteins and other fluorescent dyes, which have fluorescence in UV and visible region, such as YFP, GFP, Hoechst, and Fluorescein. The fluorescence in long wave length have advantage of optical transparency in living tissue and of low photo toxicity to cells.


CaTM-2™ AM Kit


Fig. 3. Multi-color imaging of HeLa cells.

Multi-color imaging of HeLa cells, expressing CFP in nuclear and YFP in golgi complexes, was performed after introducing of CaTM-2™ AM. CaTM-2™ is usable for multi-color imaging in various conditions, such as the cells in which fluorescent proteins are expressed in organella or calcium imaging in the cell labeled by other fluorescent probes.

Code No.	Product	Size	Remarks
GC501	CaTM-2™	500μg	Calcium probe with red fluorescence
GC502	CaTM-2™	50μg × 10	Subdivided packaging.
GC5021	CaTM-2™	50μg × 5	Small size for trial.
GC503	CaTM-2™ AM	500μg	Easy type to introduce in cell. For live-cell imaging
GC504	CaTM-2™ AM	50μg × 10	Subdivided packaging. For live-cell imaging.
GC5041	CaTM-2™ AM	50μg × 5	Small size for trial.
GC505	CaTM-2™ AM Kit	5 plates	Ready to Use Kit
GC506	CaTM-2™ potassium salt	500μg	CaTM-2™ soluble to water
GC401	CaSiR-1™	1mg	Calcium probe with fluorescence in near infra-red region
GC402	CaSiR-1™	50μg × 20	Subdivided packaging.
GC4021	CaSiR-1™	50μg × 10	Small size for trial.
GC403	CaSiR-1™ AM	50μg × 20	For live-cell imaging
GC4031	CaSiR-1™ AM	50μg × 10	Small size for trial.
GC404	CaSiR-1™ AM Kit	5 plates	Ready to Use Kit
GC405	CaSiR-1™ potassium salt	500μg	CaSiR-1™ soluble to water

References

1. Egawa T., Hirabayashi K., Koide Y., Kobayashi C., Takahashi N., Mineno T., Terai T., Ueno T., Komatsu T., Ikegaya Y., Matsuki N., Nagano T., Hanaoka K. Angew. Chem. Int. Ed. 2013, 52, 3874 -3877.
2. Egawa, T.; Hanaoka, K.; Koide, Y.; Ujita, S.; Takahashi, N.; Ikegaya, Y.; Matsuki, N.; Terai, T.; Ueno, T.; Komatsu, T.; Nagano, T. J. Am. Chem. Soc. 2011, 133, 14157-14159.
3. Mika Mizunuma, Hiroaki Norimoto, Kentaro Tao, Takahiro Egawa, Kenjiro Hanaoka, Tetsuya Sakaguchi, Hiroyuki Hioki, Takeshi Kaneko, Shun Yamaguchi, Tetsuo Nagano, Norio Matsuki & Yuji Ikegaya, Nature Neuroscience 17, 503–505 (2014), "Unbalanced excitability underlies offline reactivation of behaviorally activated neurons"

GlycoFluor™ Series

GlycoYELLOW™-βGal

Easier than X-Gal staining. For the fluorescent detection of lacZ expression within live cells, or for the observation of SA-β-Gal as an aging maker.

GlycoYELLOW™-βGal is a specific fluorescent probe for detection of β-galactosidase. It can be applied to fluorescent imaging and selection of cell and tissue transfected with lacZ.
Because GlycoYELLOW™-βGal is almost non-fluorescent in the absence of β-galactosidase, this probe exhibits good S/N ratio. With this fluorescent probe, you don't need cell fixation which is necessary by the X-Gal staining. Live cell imaging of aging marker SA-β-Gal is possible by using this probe.
(* Also usable for the fixed cells.

- **Usable for the live cell imaging**
- **High sensitivity**
- **Superior retentivity in cells**

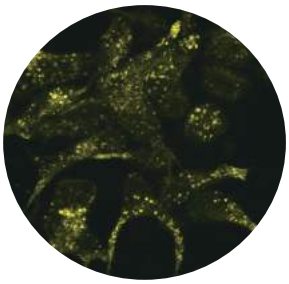


Fig. 1. Imaging of β-galactosidase activity inside HEK293 cells using GlycoYELLOW™-βGal.

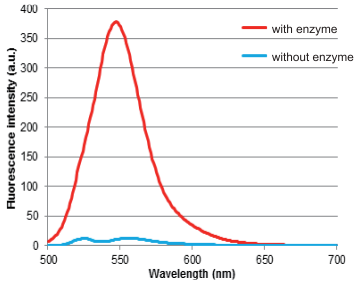


Fig. 2. Fluorescent spectra of GlycoYELLOW™-βGal before and after the reaction with β-galactosidase.

GlycoYELLOW™-βGal was incubated for 30 min with 3 units of β-galactosidase at 37°C. After the reaction with β-galactosidase, fluorescent intensity around the peak at 547 nm greatly increased about 37 times by 3 units of the enzyme.

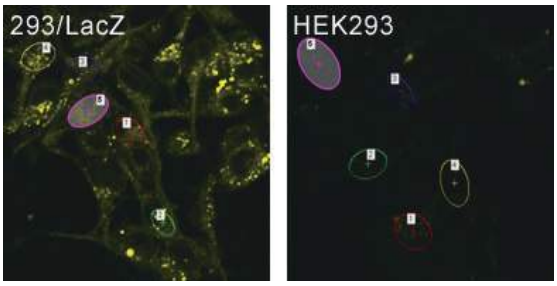


Fig. 3. Live cell imaging of 293/LacZ (β-galactosidase expressing cell line) and HEK293 cell by using GlycoYELLOW™-βGal.

Comparing these cell lines, strong fluorescence derived from GlycoYELLOW™-βGal was observed inside 293/LacZ cell.

Code No.	Product	Size	Remarks
GC601	GlycoYELLOW™-β Gal	50μg × 10	Fluorescent probe for the detection of galactosidase. For the live-cell analysis of lacZ gene.
GC602	GlycoYELLOW™-Glu	50μg × 10	Fluorescent probe for the detection of glucosidase.
GC603	GlycoYELLOW™-βGlcU	50μg × 10	Fluorescent probe for the detection of glucuronidase.
GC1001	EsterYELLOW™-Ac	50μg × 10	For the living conformation of cells.

YouTube GoryoChemical http://youtu.be/3dUeUfdzPqQ

Detection of the intracellular SA-β-Gal by GlycoYELLOW™-βGal in live cells after UV irradiation.

References

1. Mako Kamiya, Daisuke Asanuma, Erina Kuranaga, Asuka Takeishi, Masayo Sakabe, Masayuki Miura, Tetsuo Nagano, and Yasuteru Urano, J. Am. Chem. Soc. 2011, 133, 12960-12963
"蛍光プローブの精密設計に基づく in vivo 迅速蛍光がんイメージング" 神谷真子、浦野泰照　実験医学 2012, Vol. 30, No. 7 (増刊), 1135-1144

※GlycoYELLOW™-βGal is referred to as HMDER-βGal in the reference paper.

TokyoGreen®-βGal

Fluorescent substrate for β-galactosidase

TokyoGreen®-βGal is permeable through the cell membrane and is fluorescent substrate (9-(4'-methoxy-2'-methylphenyl)- 6-(β-D-galactopylanosyloxy) -xanthen-3-one) for detecting β-galactosidase. Cell lysis or fixation is not need since TokyoGreen®-βGal is taken into the cell. TokyoGreen®-βGal is applicable to the experiments of gene targeting and the cell cloning by using lacZ as gene marker.

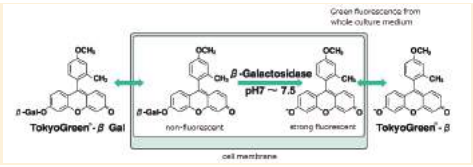
- **TokyoGreen®-βGal is recently-developed fluorescent chemical detecting the activity of β-galactosidase in living cells.**
- **TokyoGreen®-βGal is permeable through the cell membrane. Cell lysis, which is necessary for the previous color reaction using ONPG, is not needed.**
- **Does not require two glycosidic cleavages as in FDG buffer ranges.**
- **Able to continue culturing cells after the measurement of the β-galactosidase activation, because the reagent is removable by replacing the culture medium for several times.**

Principle of the measurement

Non-fluorescent TokyoGreen®-βGal is taken into the cell, is hydrolyzed by the β-galactosidase, and generates bright fluorescent TokyoGreen®. TokyoGreen® is also permeable through the cell membrane, so that generated TokyoGreen® diffuses uniformly in the culture medium and that the whole medium makes green fluorescence (510 nm) when it is irradiated by the 490 nm excitation light.

Contents

TokyoGreen®-βGal 1mg (5 mM in DMSO 0.4mL)
$C_{27}H_{26}O_9$ Mw:494.49



Code No.	Product	Size	Storage
SK4001-01	TokyoGreen-β Gal [1)]	1mg	-20℃

[1)] Dissolved in DMSO

References

1. Y. Urano, M. Kamiya, K. Kanda, T. Ueno, K. Hirose, T. Nagano: J. Am. Chem. Soc. 127, 4888-4894 (2005).
2. T. Nagano, Y. Urano, M. Kamiya "Bioimaging and chemicalbiology" Saibo-kogaku (in Japanese) 24(11), 1187-1191 (2005).

GlycoFluor™ Series

TokyoGreen®-βGlu

Fluorescent substrate for β-glucosidase

TokyoGreen®-βGlu is permeable through the cell membrane and is fluorescent substrate [9- (4'-methoxy-2'-methylphenyl) -6- (β-D-glucopylanosyloxy) -xanthen-3-one] for detecting β-glucosidase. Non-fluorescent TokyoGreen®-βGlu is hydrolyzed by the β-glucosidase, and generates bright fluorescent TokyoGreen®.

- **TokyoGreen®-βGlu is recently-developed fluorescent chemical detecting the activity of β-glucosidase with high sensitivity.**
- **TokyoGreen®, generated by hydrolysis of TokyoGreen®-βGlu, has strong fluorescence under the wide range of neutral and basic pH condition (left figure).**
- **Fluorescent intensity in in proportional to the activity of β-glucosidase (right figure).**

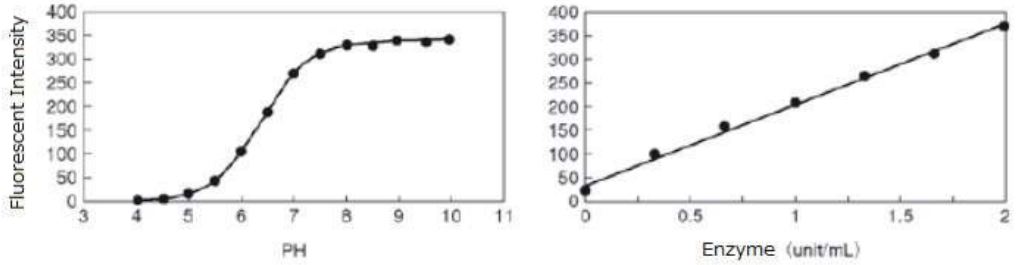


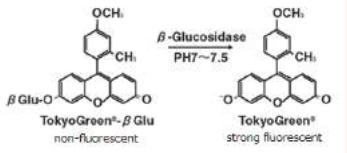
Fig. 1. Fluorescent intensity (Ex. 492 nm、Em. 510 nm) was measured 500 sec after adding β-Glucosidase (Almond) to TokyoGreen®-β Glu 10 μM in Phosphate Buffer (pH7.0).

Principle of the measurement

Non-fluorescent TokyoGreen®-βGlu is hydrolyzed by the β-galactosidase, and generates bright fluorescent TokyoGreen®. TokyoGreen® has bright green fluorescence (510 nm) when it is irradiated by the 490 nm excitation light.

Contents

TokyoGreen®-βGlu 1mg (5 mM in DMSO 0.4mL)
$C_{27}H_{26}O_9$ Mw：494.49



Code No.	Product	Size	Storage
SK4002-01	TokyoGreen-βGlu [1)]	1mg	-20℃

[1)] Dissolved in DMSO

Reference

1. Y. Urano, M. Kamiya, K. Kanda, T. Ueno, K. Hirose, T. Nagano: J. Am. Chem. Soc. 127, 4888-4894 (2005).

TokyoGreen®-βGlcU(Na)

Fluorescent substrate for β-glucuronidase

TokyoGreen®-βGlcU(Na) is permeable through the cell membrane and is fluorescent substrate [9- (4-methoxy-2-methylphenyl) -6-oxo-6Hxanthen-3-yl-β-D-glucuronide, sodium salt] for detecting β-glucuronidase. Non-fluorescent TokyoGreen®-βGlcU(Na) is hydrolyzed by the β-glucuronidase, and generates bright fluorescent TokyoGreen®.

- **TokyoGreen®-βGlcU(Na) is recently-developed fluorescent chemical detecting the activity of β-glucuronidase with high sensitivity.**
- **TokyoGreen®, generated by hydrolysis of TokyoGreen®-βGlcU(Na), has strong fluorescence under the wide range of neutral and basic pH condition (left figure).**
- **Fluorescent intensity in in proportional to the activity of β-glucuronidase (right figure).**

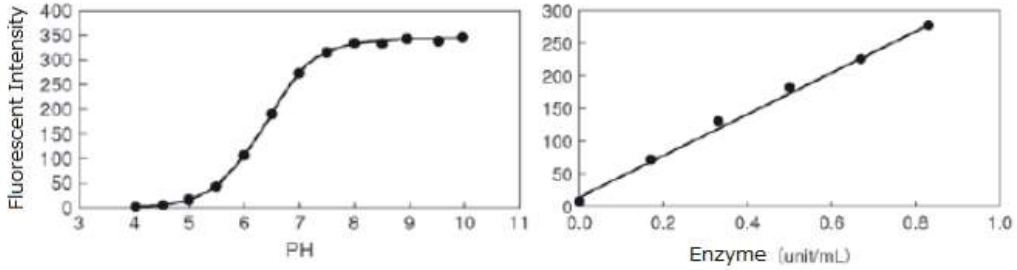


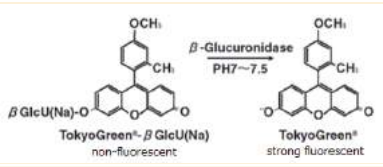
Fig. 1. Fluorescent intensity (Ex. 492 nm, Em. 510 nm) was measured 500 sec after adding β-Glucuronidase (Escherichia coli, Type IX-A) in Phosphate Buffer (pH7.0) containing TokyoGreen®-βGlcU(Na) 5 μM .

Principle of the measurement

Non-fluorescent TokyoGreen®-βGlcU(Na) is hydrolyzed by the β-glucuronidase, and generates bright fluorescent TokyoGreen®. TokyoGreen® has bright green fluorescence (510 nm) when it is irradiated by the 490 nm excitation light.

Contents

TokyoGreen®-βGlcU(Na) 1mg (5 mM in DMSO 0.38mL)
$C_{27}H_{23}NaO_{10}$ Mw:530.46



Code No.	Product	Size	Storage
SK4003-01	TokyoGreen-βGlcU(Na) [1)]	1mg	-20℃

[1)] Dissolved in DMSO

Reference

1. Y. Urano, M. Kamiya, K. Kanda, T. Ueno, K. Hirose, T. Nagano: J. Am. Chem. Soc. 127, 4888-4894 (2005).

Fuorescent probe for the selective detection of cancer cells

ProteoGREEN™-gGlu

Human cancer cells, such as hepatoma, ovarian cancer, and carcinoma, express high amount of GGT (γ-glutamyltranspeptidase) on the surface of their cell membrane. ProteoGreenTM-gGlu, non-fluorescent before the reaction, selectively makes green fluorescence by the GGT activity. Thus, cancer cells, expressing overexpressing GGT, are specifically detected by the probe. The Fluorescent intensity is amplified by the turnover of the enzyme, which increases S/N ratio.

- **Detecting cancer cells with GGT activity**
- **Fluorescent imaging of cancer cells specifically**
- **Usable for live-cell imaging (by 488 nm excitation)**

Fig. 2. (Background image) Reaction of ProteoGREEN™-gGlu with GGT
The fluorescent intensity increases over 350 times under the physiological condition (pH 7.4), after activation by GGT.
Mechanism of reaction of ProteoGREEN™-gGlu with GGT
ProteoGREEN™-gGlu selectively reacts on the human ovarian cancer, in which GGT is highly expressed, and make fluorescence.

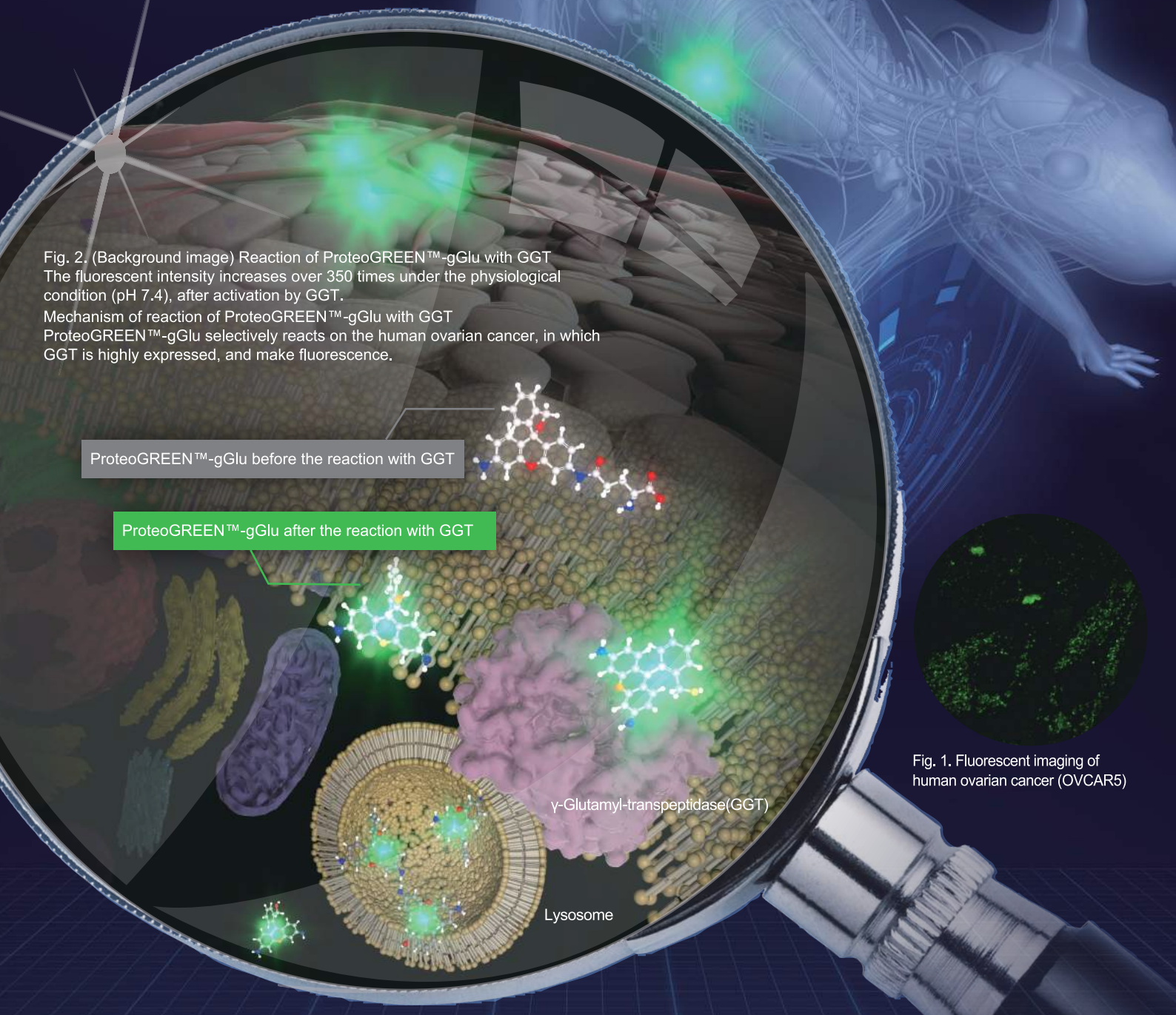
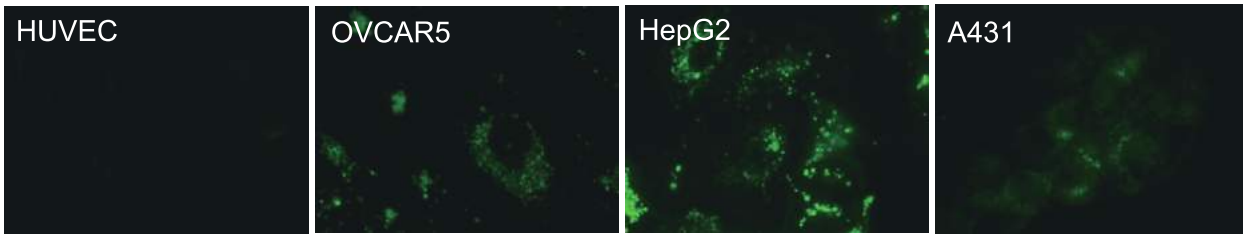


Fig. 1. Fluorescent imaging of human ovarian cancer (OVCAR5)

Application example　ProteoGREEN™-gGlu



HUVEC : Human umbilical vein endothelial cell　OVCAR5 : Human ovarian cancer　HepG2 : Human hepatoma　A431 : Human carcinoma

Fig. 3. Live cell imaging of various cancer cells and HUVEC (normal cell).
Each cell was observed by fluorescent microscopy after addition of 2 μM of ProteoGREEN™-gGlu followed by 1 h incubation. Only cancer cells produced fluorescence.

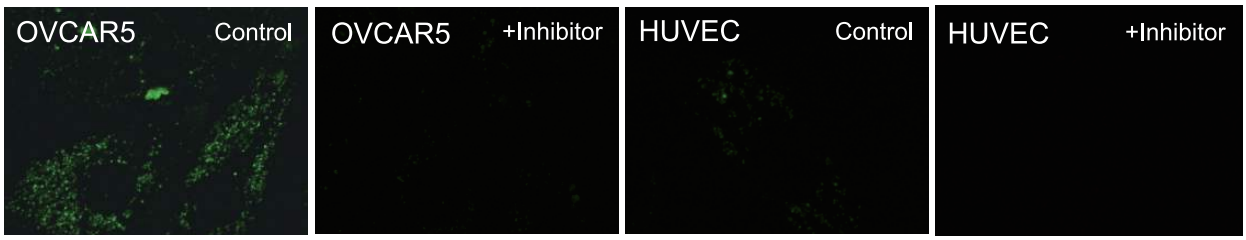


Fig. 4. Inhibition by the GGT selective inhibitor.
Intensity of the fluorescence was decreased by the hydrophilic GGT selective inhibitor, indicating that hydrophobic product of ProteoGREEN™-gGlu was taken into the cell, which make the cancer cell fluorescent.

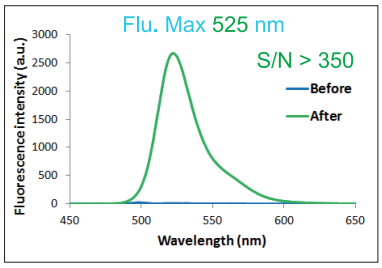


Fig. 5.Change in the fluorescence of ProteoGREEN™-gGlu by the GGT.
The probe makes fluorescence 350 times brighter by GGT. It is excited by 488 nm.

Ⅰ. Cancer cells that ProteoGREEN™-gGlu was reported to be used for their studies

derivation	
SHIN3 cells	ovarian cancer
SKOV3 cells	ovarian cancer
OVCAR3 cells	ovarian cancer
OVCAR4 cells	ovarian cancer
OVCAR5 cells	ovarian cancer
OVCAR8 cells	ovarian cancer
A2780 cells	ovarian cancer
A2780PTX22 cells	ovarian cancer
IGR-OV1 cells	ovarian cancer
Hey-A8 cells	ovarian cancer
CaOV3 cells	ovarian cancer
A549 cells	pulmonary neoplasm
HuCCT1 cells	cholangiocarcinoma
HepG2 cells	hepatoma
colitis-associated colon cancer	

Ⅱ. Cancer cells that GGT activity was reported to be high.

derivation	
HL60 cells	leukemia cell
U937 cells	lymphoma

Ⅲ. Cancer cells that GGT activity was reported to be moderate.

derivation	
A431 cells	carcinoma

Ⅳ. Normal cell with low GGT activity

derivation	
HUVEC	Human umbilical vein endothelial cell

＊Please feel free to ask any questions.

Observation method

Excitation light of wave length of 488 nm is ideal. Long-pass filter such as GFP-LP (Nikon) or U-MWB2 (Olympus) is suitable for the observation. Peak of the fluorescent wave length appears around 525 nm.

Code No.	Product	Size	Remarks
GC801	ProteoGREEN™-gGlu	20μg × 10	Usable not only for fluorescent imaging of cancer cells expressing high amount of GGT, and for FACS analysis, but also in vivo or ex vivo imaging.

References

1. Urano, Y.; Sakabe, M.; Kosaka, N.; Ogawa, M.; Mitsunaga, M.; Asanuma, D.; Kamiya, M.; Young, M. R.; Nagano, T.; Choyke, P. L.; Kobayashi, H. Sci. Transl. Med. 2011, 129, 1-10.
2. Mitsunaga, M.; Kosaka, N.; Choyke, PL.; Young, MR.; Dextras, CR.; Saud, SM.; Colburn ,NH.; Sakabe, M.; Nagano, T.; Asanuma, D.; Urano, Y.; Kobayashi, H. Gut., 2013, 62, 1179-1186.
3. in vivo がん検出を可能とする蛍光有機小分子プローブの開発
浦野泰照、神谷真子　病理と臨床　30(7): 747-754, 2012
4. 蛍光プローブの精密設計による高精細in vivo癌イメージング
浦野泰照　分子消化器病　9(2):138-144, 2012
5. 新規蛍光プローブによるin vivo微小がん検出の実現
浦野泰照　癌と化学療法　40(3): 299-303, 2013
6. 蛍光プローブの精密設計による新しい生細胞イメージング・in vivoがんイメージング
浦野泰照、神谷真子　実験医学　30(15): 2519-2526, 2012

＊ProteoGREEN™-gGlu is referred to as gGlu-HMRG in the reference paper 1.

ProteoFluor™ Series

ProteoGREEN™-peptides

Bright fluorescent enzyme substrate with long wave length.
For live-cell imaging or in vitro assay.
Custom synthesis of fluorescent enzyme substrates.

HMRG (Hydroxyl Methyl Rhodamine Green) is fluorescent parent compound of ProteoGREEN™-gGlu. By using HMRG as a fluorescent parent compound, we are able to synthesize fluorescent enzyme substrates 30 times brighter than MCA (methylcoumarin amide) and high S/N ratio because of high fluorescent intensity and no background fluorescence before the enzymatic reaction.

- **Visualizing various enzymatic reactions by the hydrolysis reaction.**
- **Dealing with many enzymes, such as protease or glycosidase.**
- **Higher enzymatic reaction and longer wavelength than MCA.**

(1) Fluorescence is emitted by the specific restriction of the enzyme recognition site (indicated in blue in the figure).

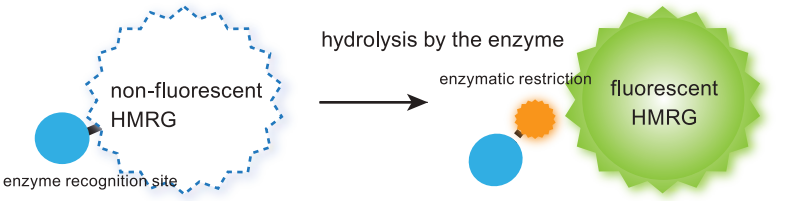


Figure: Hydrolysis reaction of enzyme and enzyme substrate.

(2) Possible to design by the novel enzymes including not only protease (caspase 3, trypsin etc.), glycosidase or galactosidase but also dipeptides or tripeptides.

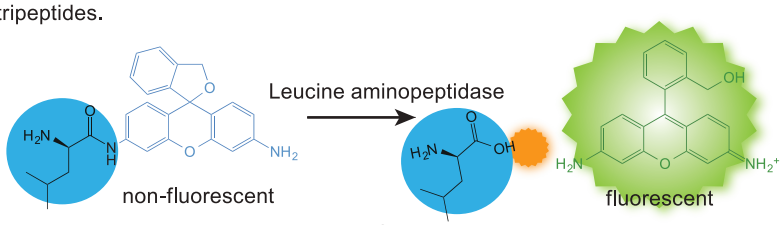
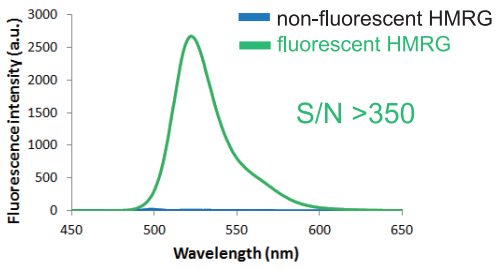


Figure: An example of leucine aminopeptidase

(3) Fluorescence from HMRG is 30 times brighter than MCA and it has no background fluorescence before the enzymatic reaction.

fluorescent probe	MCA	HMRG
excitation wave length	380nm	488nm
emission wave length	460nm	525nm
cell permeability	unknown	yes
photo toxicity	high because of short fluorescent wave length	low because of long fluorescent wave length
fluorescence quantum yield	~0.3	~0.8
ε	30,000 ~ 40.000	70,000 ~ 80.000



References

1. Masayo Sakabe, Daisuke Asanuma, Mako Kamiya, Ryu J. Iwatate, Kenjiro Hanaoka, Takuya Terai, Tetsuo Nagano, and Yasuteru Urano, J. Am. Chem. Soc., 2013, 135 (1), pp 409–414., “Rational Design of Highly Sensitive Fluorescence Probes for Protease and Glycosidase Based on Precisely Controlled Spirocyclization”
2. Urano, Y.; Sakabe, M.; Kosaka, N.; Ogawa, M.; Mitsunaga, M.; Asanuma, D.; Kamiya, M.; Young, M. R.; Nagano, T.; Choyke, P. L.; Kobayashi, H. Sci. Transl. Med. 2011, 129, 1-10.
3. 蛍光プローブの精密設計による新しい生細胞イメージング・in vivoがんイメージング、浦野泰照、神谷真子　実験医学　30(15): 2519-2526, 2012

※ProteoGREEN™-gGlu is referred to as gGlu-HMRG in the reference papers.

We synthesize fluorescent enzyme substrates as you like. Please feel free to ask us on info@polaris-t.com.

Ascertainment of iPS/ES cells

Chemical probe for human iPS/ES cells

Kyoto Probe 1 (KP-1)

Able to distinguish human iPS/ES cells from differentiated cells

Usable for flow cytometry or live cell imaging

Dyeing while culturing

Feature 1.

Able to distinguish human iPS/ES cells from differentiated cells

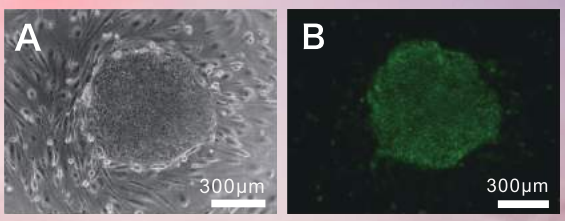
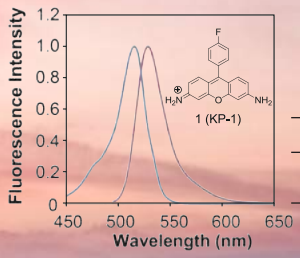


Fig.1. (A, B) Images of iPS colony, formed on the feeder cells, stained with KP-1. (A: bright field image, B: fluorescent image).



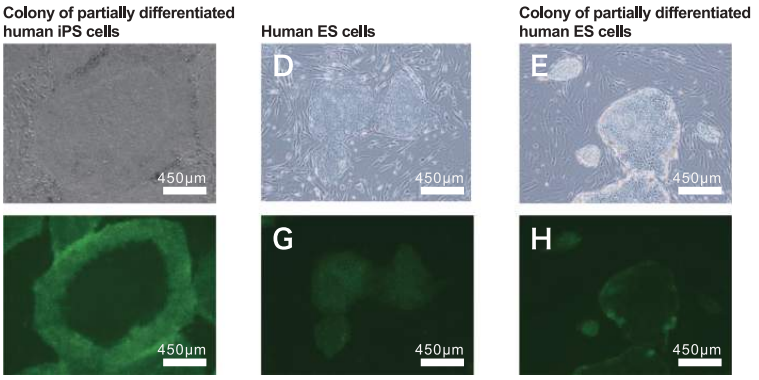
λ_{ex}(nm)	λ_{em}(nm)	ϕ
515	529	0.45

Fig.2. Fluorescent feature of KP-1.

Feature1 (Continued)

Able to distinguish human iPS/ES cells from differentiated cells

Fig.3. (C, F) Images of partially differentiated human iPS cell colony stained with KP-1. (C: bright field image, F: fluorescent image). Surrounding region of the colony, in which cells were not differentiated, were specifically stained. (D, G) Images of a colony of human ES cells stained with KP-1. (D: bright field image, G: fluorescent image). (E, H) Images of partially differentiated human ES cell colony stained with KP-1. (E: bright field image, H: fluorescent image). Differentiated cells were not stained.



Feature2

Usable for flow cytometry or live cell imaging

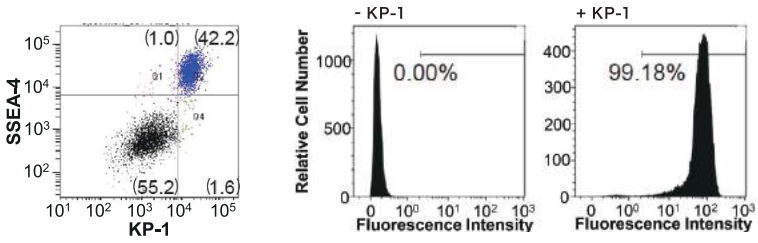
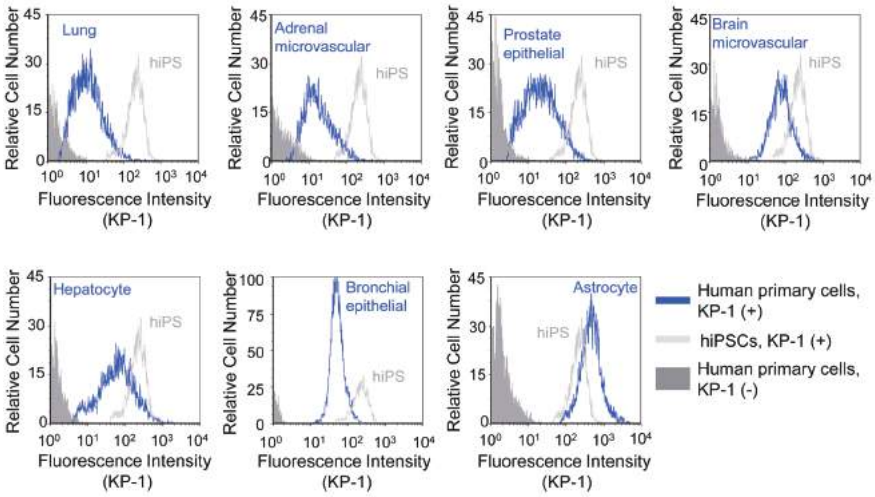


Fig.4. FACS analysis of human iPS cells (Left panel) When the mixture of human iPS cells and feeder cells were analyzed, most SSEA-4, pluripotent stem cell marker, positive cells were also positive to KP-1. SSEA-4 is a well-known cell surface marker of pluripotent stem cell. (Center and Right panel) 99.18% of human iPS cells were stained by KP-1.

Feature3

Isolation of human iPS cells by flow cytometry.

Fig.5. Flow cytometry to various somatic cells with KP-1.
When analyzed by flow cytometry with KP-1 probe, human iPS cells are able to be isolated from various somatic cells, except for nerve cell.



Code No.	Product	Outline	Size
GC7001-01	Kyoto Probe 1 (KP-1)	Probe for detecting human iPS/ES cells	10μg × 5
GC7001-02	Kyoto Probe 1 (KP-1)	Probe for detecting human iPS/ES cells	10μg × 10

Reference

1. Hirata N, Nakagawa M, Fujibayashi Y, Yamauchi K, Murata A, Minami I, Tomioka M, Kondo T, Kuo TF, Endo H, Inoue H, Sato S, Ando S, Kawazoe Y, Aiba K, Nagata K, Kawase E, Chang YT, Suemori H, Eto K, Nakauchi H, Yamanaka S, Nakatsuji N, Ueda K, Uesugi M. "A Chemical Probe that Labels Human Pluripotent Stem Cells" Cell Rep. 2014 6:1165-1174.

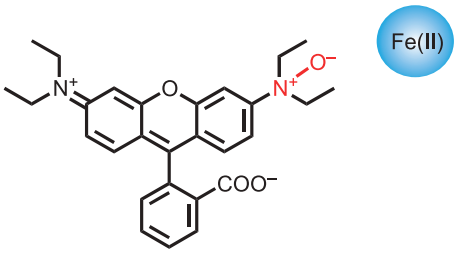
MetalloFluor™ Series

FeRhonox™-1

Detecting iron(II) ion in live cell imaging

Iron is the most abundant transition metal in our body, acts as oxygen carrier or active center of various enzymes and is indispensable for our life. On the other hand, its strong oxidation-reduction activity has a toxic effect on the cells, and the excess existence of iron causes cell death. Actually, iron overload is suggested carcinogenicity, and iron ion deposition is observed in neurodegenerative disease, indicating the relation between diseases and homeostasis of iron. FeRhonox™-1 is a fluorescent probe detecting ferrous iron that contributes to the generation of reactive oxygen species (ROS) in vivo.

- **Specifically detecting ferrous iron**
- **Applicable to live cell imaging of free iron(II) ion**
- **Detecting iron(II) ion in Golgi complex**



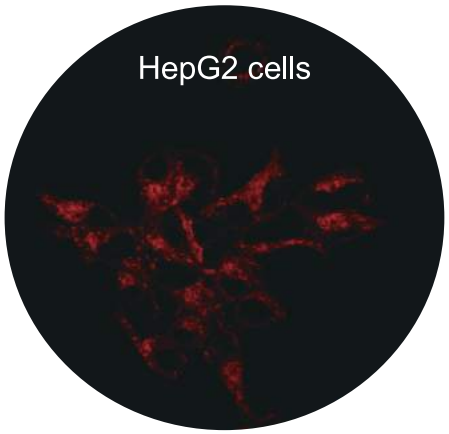


Fig. 1. Fluorescent live-cell imaging of iron(II) ion by FeRhonox™-1.

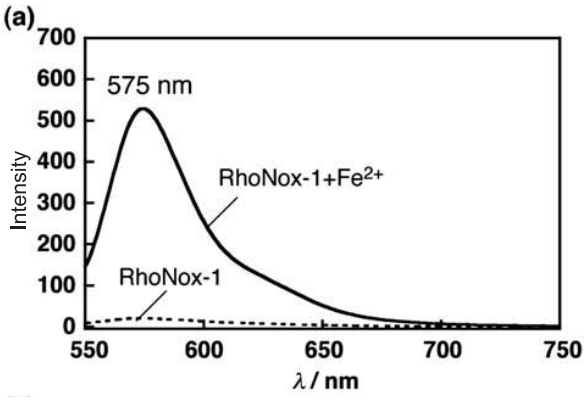


Fig. 2. Response of FeRhonox™-1 to iron(II) ion.
Fluorescent intensity increased upon the addition of iron(II) ion.

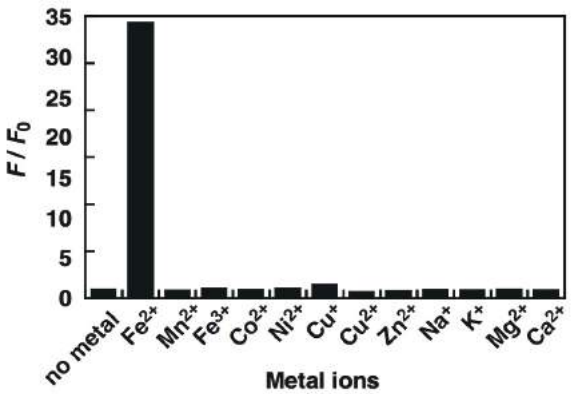


Fig. 3. Fluorescent intensity of FeRhonox™-1 with various metal ion.
FeRhonox™-1 reacted specific to iron(II) ion.

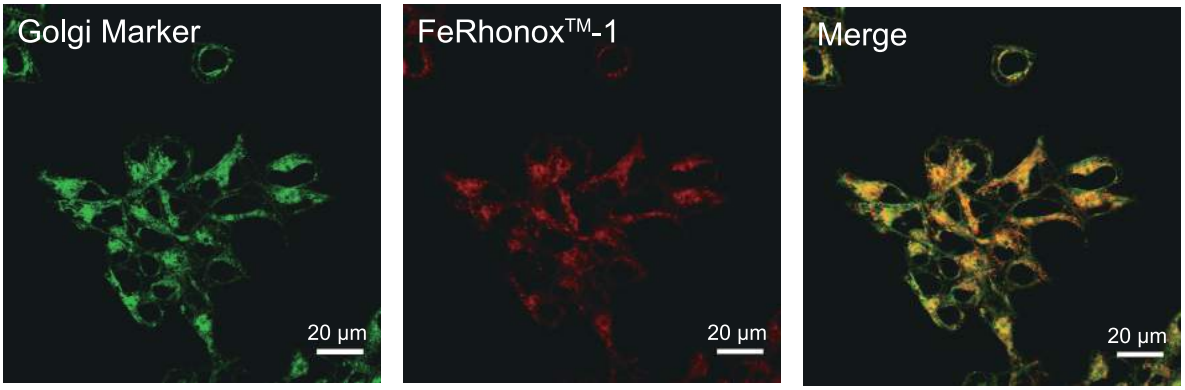
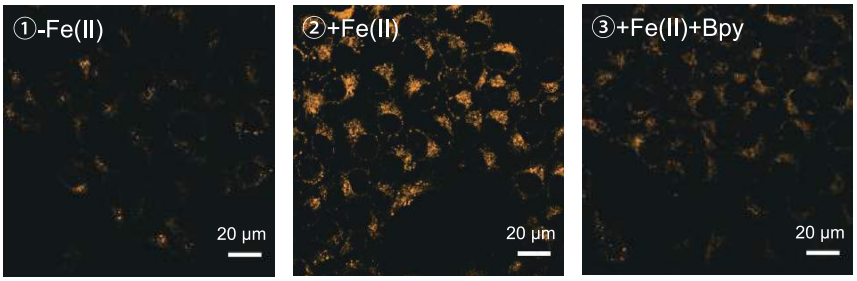


Fig. 4. Images of HepG2 cell subjected to multiple staining by Golgi Marker and FeRhonox™-1.
Merged image indicated that FeRhonox™-1 selectively detected iron(II) ion in Golgi complex.

Application example FeRhonox™-1

Application to imaging of HepG2 cell.



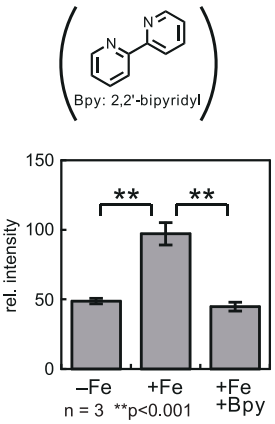
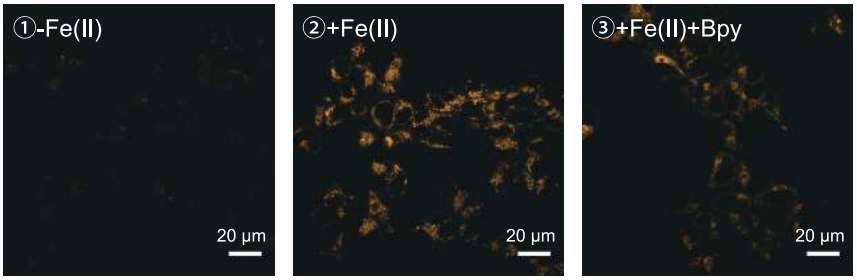


Fig. 5. Live imaging of HepG2 cell stained by FeRhonox™-1.
(1) Without addition of iron(II) ion. (2) Adding iron(II) ion (100 μM Ferrous ammonium sulfate). (3) Adding both iron(II) ion and iron chelator Bpy. Fluorescent intensity of FeRhonox™-1 increased by addition of iron(II) ion in (2). However, it decreased in (3), suggesting that FeRhonox™-1 specifically detected iron(II) ion in the cell.

Application to imaging of HEK293 cell.



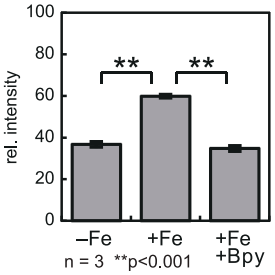


Fig. 6. Live imaging of HEK293 cell stained by FeRhonox™-1.
(1) Without addition of iron(II) ion. (2) Adding iron(II) ion. (3) Adding both iron(II) ion and Bpy. Fluorescent intensity of FeRhonox™-1 increased by addition of iron(II) ion in (2). However, it decreased in (3), suggesting that FeRhonox™-1 specifically detected iron(II) ion.

Detection of intracellular ferrous ion.

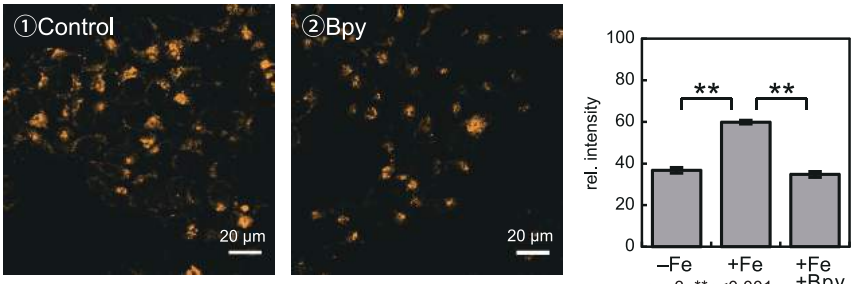


Fig. 7. Imaging of intracellular iron(II) ion.
Fluorescent intensity decreased upon addition of Bpy, indicating that FeRhonox™-1 specifically detected intracellular iron(II) ion.
＊Bpy: 2,20-bipyridyl (Bpy) is permeable through the cell membrane and a selective chelator of iron(II) ion.

Observation method

Green fluorescence (throgh the fluorescence filter G-1A (Nikon), U-FGW (Olympus) or N2.1 (Leica) with excitation 546 nm) suites for the excitation light. Observation was performed after staining live cells by 1 h incubation with 5 μM of the dye at 37°C. Peak of the fluorescence appears around 575 nm.

Code No.	Product	Size	Remarks
GC901	FeRhonox™-1	50μg × 10	Fluorescent probe for detecting iron(II) ion

References

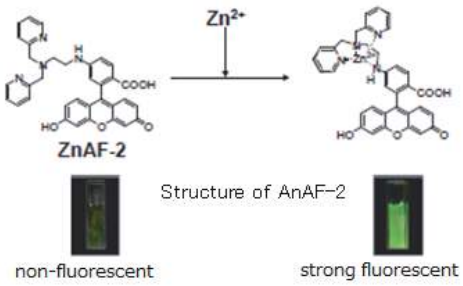
1. Hirayama, T.; Okuda, K.; Nagasawa, H. Chem. Sci. 2013, 4, 1250-1256.
2. Mukaide, T.; Hattori, Y.; Misawa, N.; Funahashi, S.; Jiang, L.; Hirayama, T.; Nagasawa, H.; Toyokuni, S. Free Radic. Res. 2014, 48, 990-995.
3. Tomoyo Imamura, Tasuku Hirayama, Kazuhiro Tsuruma, Masamitsu Shimazawa, Hideko Nagasawa, Hideaki Hara, Volume 129, December 2014, Pages 24–30, Experimental Eye Research "Hydroxyl radicals cause fluctuation in intracellular ferrous ion levels upon light exposure during photoreceptor cell death"

MetalloFluor™ Series

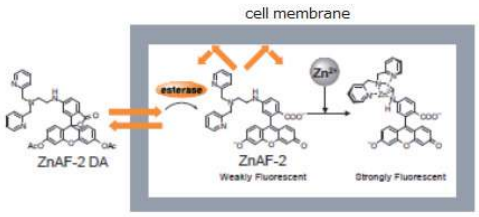
ZnAF-2 / ZnAF-2 DA

Fluorescent probe for detecting zinc ion

ZnAF-2, that has structure of fluorescein connected with TPEN analog, has high specificity for Zn^{2+}.



ZnAF-2 has high solubility, so that it is not permeable through the cell membrane. ZnAF-2 DA has an additional acetyl group which make the reagent go inside the cell much easier, and the acetyl group is removed by the intracellular esterase which make the reagent stay inside the cell for long time.



- **High affinity for Zn^{2+} that enables us to detect low concentration of Zn^{2+} (dissociation constant: 2.7 nM).**
- **High specificity for Zn^{2+} in the buffer.**
- **Low background fluorescence contributes high sensitivity of Zn^{2+} in living sample.**
- **ZnAF-2 DA is permeable through the cell membrane, so that by using the reagent, bioimaging in living cell or tissue is available.**
- **ZnAF-2 DA is deacetylated by esterase in the cell, and it stays inside the cell for long time.**

Principle of the measurement

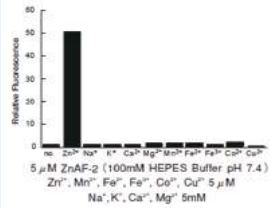
Complex of ZnAF-2 and Zn^{2+} has green fluorescence (wavelength of 515 nm) by the excitation at 492 nm.

Contents

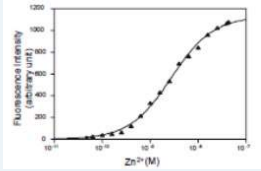
ZnAF-2 $C_{34}H_{32}Cl_4N_4O_5$	1mg (DMSO 0.28mL) Mw：718.45
ZnAF-2 DA $C_{38}H_{32}N_4O_7$	1mg (DMSO 0.28mL) Mw：656.23

Preparation of the reagent

Density of the provided sample is 5mmol/L in DMSO. Dilute 1000 times with neutral buffer before use.

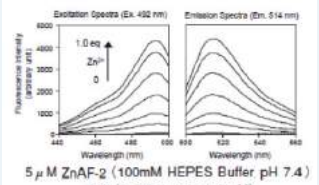


Fluorescent intensity of ZnAF-2 with various metal ion.



1μM ZnAF-2
(100mM HEPES Buffer pH 7.4)

Fluorescence intensity of ZnAF-2 with Zn^{2+}.



5 μM ZnAF-2 (100mM HEPES Buffer pH 7.4)
Zn^{2+} (0, 0.5, 1, 2, 3, 4, 5 μM)

Code No.	Product	Size	Storage
SK2001-01	ZnAF-2 [1)]	1mg	2~10℃
SK2002-01	ZnAF-2 DA [1)]	1mg	-20℃

[1)] Dissolved in DMSO

References

1. Hirano, T.; Kikuchi, K.; Urano, Y.; Higuchi, T.; Nagano, T.J. Am. Chem. Soc. 2000, 122, 12399-12400
2. Hirano, T.; Kikuchi, K.; Urano, Y.; Nagano, T. J. Am.Chem. Soc. 2002, 124, 6555-6562
3. Ueno, S.; Tsukamoto, M.; Hirano, T.; Kikuchi, K.; Yamada,MK.; Nishiyama, N.; Nagano, T. Matsuki, N.; Ikegaya, Y.J. Cell. Biol. 2002, 158, 215-220

Fluorescent probes for detecting nitrogen monoxide (NO)

NOFluor™ Series

Diaminofluorescein-2(DAF-2)

- **Real time observation of NO generated by tissues or cells is available.**
- **Low autofluorescence from the living sample because Diaminofluorescein-2 (DAF-2) is excited by the visible light.**
- **Low damage to the cells because Diaminofluorescein-2 (DAF-2) is excited by the visible light.**
- **Needless to change pH for the measurement because Diaminofluorescein-2 (DAF-2) captures NO and makes fluorescence under neutral pH condition.**
- **High sensitivity and selectivity.**

Principle of the measurement

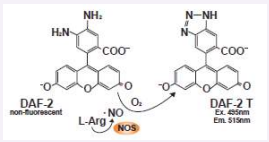
Amino groups of Diaminofluorescein-2 (DAF-2) capture NO, and the DAF-2 makes fluorescence of green light with wave length of 515 nm when it's excited by the light with wavelength of 495 nm.

Contents

DAF-2 1mg (in 0.55 mL DMSO)
$C_{20}H_{14}N_2O_5$ Mw:362.3

Preparation of the reagent

Concentration of the provided sample is 5mmol/L in DMSO. Dilute 500 times with neutral buffer before use.



Code No.	Product	Size	Storage
SK1001-01	Diaminofluorescein-2 (DAF-2) [1]	1mg	-20℃

[1] Dissolved in DMSO

References

1. Kojima, H., Nakatsubo, N., Kikuchi, K., Kawahara, S., Kirino, Y., Nagoshi, H., Hirata, Y., and Nagano, T. Anal. Chem. 70 2446-2453, 1998.
2. Kojima, H. and Nagano, T. Jikken Igaku (in Japanese) Vol.17, No.8 946-950(1999)
3. Kojima, H., Nakatsubo, N., Kikuchi, K., Kawahara, S., Kirino, Y., Nagoshi, H, Hirata, Y, Maeda D,Imai Y,Irimura T and Nagano, T. FEBS Letters,427,263-266, 1998.

Diaminofluorescein-2 diacetate(DAF-2 DA)

- **Permeable through the cell membrane and real time observation of NO inside tissues or cultured cells is available.**
- **Long time localization inside the cell.**
- **Easily observed by a fluorescence microscopy.**
- **Low damage to the cells because the reagent is excited by the visible light.**
- **High sensitivity and selectivity.**

Principle of the measurement

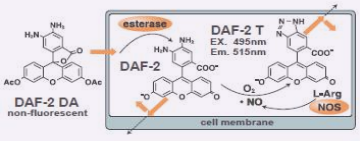
After the permeation through the cell membrane, Diaminofluorescein-2 diacetate (DAF-2 DA) is hydrolyzed by intracellular esterase to Diaminofluorescein-2 (DAF-2) which is unpermeable through the cell membrane. Amino groups of Diaminofluorescein-2 (DAF-2) capture NO, and the DAF-2 makes fluorescence of green light with wave length of 515 nm when it's excited by the light with wavelength of 495 nm.

Contents

DAF-2 DA 1mg (in DMSO 0.45mL)
$C_{24}H_{18}N_2O_7$ Mw:446.4

Preparation of the reagent

Concentration of the provided sample is 5mmol/L in DMSO. Dilute 500 times with neutral buffer before use.



Code No.	Product	Size	Storage
SK1002-01	Diaminofluorescein-2 diacetate (DAF-2 DA) [1]	1mg	-20℃

[1] Dissolved in DMSO

References

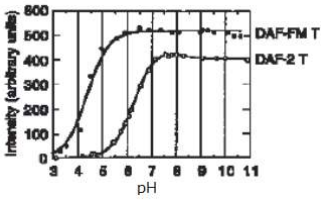
1. Nagano, T. and Kojima, H. Gendaikagaku (in Japanese) 9, No.342.23-30(1999)
2. Kojima, H., Nakatsubo, N., Kikuchi, K., Kawahara, S., Kirino, Y., Nagoshi, H., Hirata, Y., and Nagano, T. Anal. Chem. 70 2446-2453, (1998)
3. Kojima, H. and Nagano, T. Jikken Igaku (in Japanese) Vol.17, No.8 946-950(1999)

Fluorescent probes for detecting nitrogen monoxide (NO)

NOFluor™ Series

Diaminofluorescein-FM (DAF-FM)

- High fluorescence intensity even at low pH (around pH 6) conditions.
- Real time observation of NO generated by tissues or cells is available.
- Low autofluorescence from the living sample because the reagent is excited by the visible light.
- Low damage to the cells because the reagent is excited by the visible light.
- Needless to change pH for the measurement because the reagent captures NO and makes fluorescence under neutral pH condition.
- High sensitivity and selectivity.



Principle of the measurement

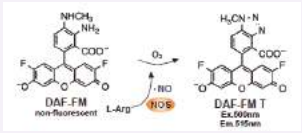
Amino groups of DAF-FM capture NO, and the DAF-FM makes fluorescence of green light with wave length of 515 nm when it's excited by the light with wavelength of 500 nm.

Contents

DAF-FM 1mg (in DMSO 0.35 mL)
$C_{21}H_{14}F_2N_2O_5$ Mw:412.34

Preparation of the reagent

Concentration of the provided sample is 7 mmol/L in DMSO. Dilute 1000 times with neutral buffer before use.



Code No.	Product	Size	Storage
SK1003-01	Diaminofluorescein-FM (DAF-FM) [1]	1mg	-20℃

[1] Dissolved in DMSO

References

1. Kojima H, Urano Y, Kikuchi K, Higuchi T, Hirata Y, Nagano T Angew Chem Int Ed 1999; 38: 3209-3212.
2. Kojima H, Hirata M, Kikuchi K, Kudo Y, Nagano T Journal of Neurochemistry,2001; 76: 1404-1410.

Diaminofluorescein-FM diacetate (DAF-FM DA)

- High fluorescence intensity even at low pH (around pH 6) conditions.
- Permeable through the cell membrane and real time observation of NO inside tissues or cultured cells is available.
- Long time localization inside the cell.
- Easily observed by a fluorescence microscopy.
- Low damage to the cells because the reagent is excited by the visible light.
- High sensitivity and selectivity.

Principle of the measurement

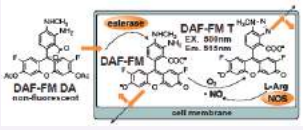
After the permeation through the cell membrane, DAF-FM DA is hydrolyzed by intracellular esterase to DAF-FM which is unpermeable through the cell membrane. Amino groups of DAF-FM capture NO, and the DAF-FM makes fluorescence of green light with wave length of 515 nm when it's excited by the light with wavelength of 500 nm.

Contents

DAF-FM DA 1mg (in DMSO 0.4 mL)
$C_{25}H_{18}F_2N_2O_7$ Mw:496.42

Preparation of the reagent

Concentration of the provided sample is 5 mmol/L in DMSO. Dilute 500 times with neutral buffer before use.



Code No.	Product	Size	Price	Storage
SK1004-01	Diaminofluorescein-FM diacetate (DAF-FM DA) [1]	1mg	Price	-20℃

[1] Dissolved in DMSO

References

1. Kojima H, Urano Y, Kikuchi K, Higuchi T, Hirata Y, Nagano T Angew Chem Int Ed 1999; 38: 3209-3212.
2. Kojima H, Hirata M, Kikuchi K, Kudo Y, Nagano T Journal of Neurochemistry,2001; 76: 1404-1410.

Diaminorhodamine-4M (DAR-4M)

- **Real time observation of NO generated by tissues or cells is available.**
- **Low autofluorescence from the living sample because DAR-4M is excited by the light with long wave length.**
- **High intensity of fluorescence at the wide range of pH (from pH 4 to 12).**

Principle of the measurement

Amino groups of DAR-4M capture NO, and it makes fluorescence of green light with wave length of 575 nm when it's excited by the light with wavelength of 560 nm.

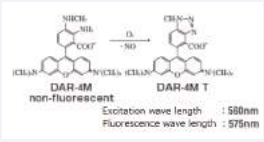
Contents

DAR-4M 1mg (in DMSO 0.47 mL)

$C_{25}H_{26}N_4O_3$ Mw:430.50

Preparation of the reagent

Concentration of the provided sample is 5 mmol/L in DMSO. Dilute 500 times with neutral buffer before use.



Code No.	Product	Size	Storage
SK1005-01	Diaminorhodamine-4M (DAR-4M) [1)]	1mg	-20℃

[1)] Dissolved in DMSO

Reference

1. Kojima, H., Hirotani, M., Nakatsubo, N., Kikuchi, K., Urano, Y., Higuchi, T., Hirata, Y., Nagano, T. Anal. Chem. 2001; 73:1967-1973

Diaminorhodamine-4M acetoxymethyl ester (DAR-4M AM)

- Permeable through the cell membrane and real time observation of NO inside tissues or cultured cells is available.
- Low autofluorescence from the living sample because the reagent is excited by the light with long wave length.
- High intensity of fluorescence at the wide range of pH (from pH 4 to 12).

Principle of the measurement

Amino groups of Diaminorhodamine react with NO, and it makes fluorescence of green light with wave length of 575 nm when it's excited by the light with wavelength of 560 nm.

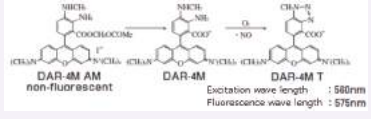
Contents

DAR-4M AM 1mg (in DMSO 0.32 mL)

$C_{28}H_{31}IN_4O_5$ Mw:630.47

Preparation of the reagent

Concentration of the provided sample is 5 mmol/L in DMSO.
Dilute 500 times with neutral buffer before use.



Code No.	Product	Size	Storage
SK1006-01	Diaminorhodamine-4M acetoxymethyl ester (DAR-4M AM) [1)]	1mg	-20℃

[1)] Dissolved in DMSO

Reference

1. Kojima, H., Hirotani, M., Nakatsubo, N., Kikuchi, K., Urano, Y., Higuchi, T., Hirata, Y., Nagano, T. Anal. Chem. 2001; 73:1967-1973

ROSFluor™ Series

NiSPY-3 (Nitrative Stress Sensing Pyrromethene Dye)

Fluorescent probe for detecting nitrosative stress

NiSPY-3 (Nitrative Stress Sensing Pyrromethene Dye) is fluorescent reagent for detecting nitrosative stress.
NiSPY-3 reacts with specifically peroxynitrite ($ONOO^-$) when it works as detection reagent for reactive oxygen species.

- NiSPY-3 reacts specifically with peroxynitrite ($ONOO^-$) among other reactive oxygen species such as $^{\cdot}OH$, $O^{\cdot-}_2$, H_2O_2, $^1O_2$, NO etc. Fluorescent intensity is not increased by the existence of hydroxyl radical, singlet oxygen, hydrogen peroxide, hypochlorite, nitric oxide or superoxide.
- Live cell fluorescent imaging is available with NiSPY-3.

Principle of the measurement

NiSPY-3 does not have fluorescence in neutral solution. When NiSPY-3 reacts with peroxynitrite, it becomes to have strong fluorescence (excitation: 490 nm, emission: 515 nm).

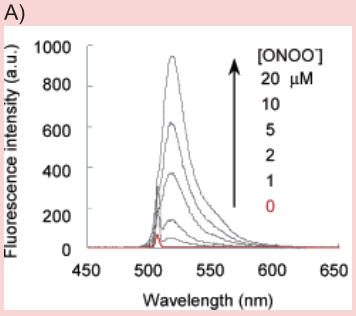
Contents

NiSPY-3 1mg
$C_{23}H_{19}BF_2N_4O_4$ Mw:464.23

Preparation of the reagent

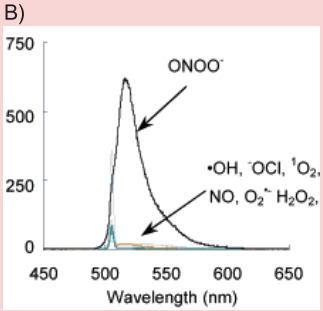
Dissolve all the reagent in 430 µL of DMSO (5mmol/L), and then dilute 500-5000 times with neutral buffer (final concentration: 1-10 mM) before use. We recommend to use up at once after probes are diluted.

A)



A)Fluorescence spectra of NiSPY-3 solution (10 µM NiSPY-3 in 0.1 M phosphate buffer pH 7.4 containing 0.1% DMF as a cosolvent) upon addition of peroxynitrite (final 0, 1, 2, 5,10, 20 µM).

B)



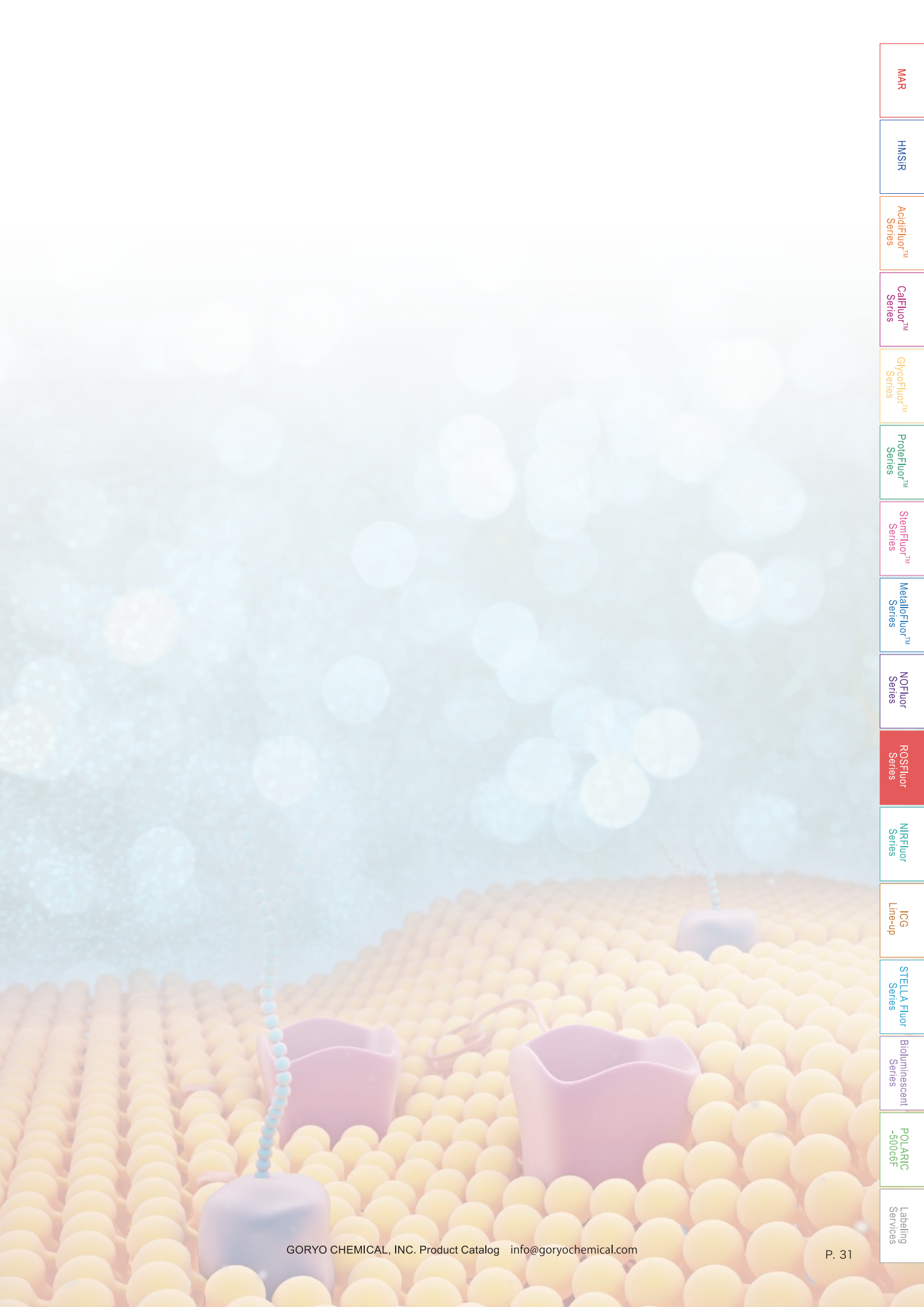
B)Fluorescence response of NiSPY-3 in various ROS generation systems

Code No.	Product	Size	Storage
SK3003-01	NiSPY-3 [1)]	1mg	-20℃

[1)] Powder

Reference

1. T. Ueno, Y. Urano, H. Kojima and T. Nagano: J. Am. Chem. Soc., 128, 10640-10641 (2006) .



ROSFluor™ Series

Hydroxyphenyl Fluorescein(HPF) / Aminophenyl Fluorescein(APF)

Fluorescent probes for detecting reactive oxygen species (ROS)

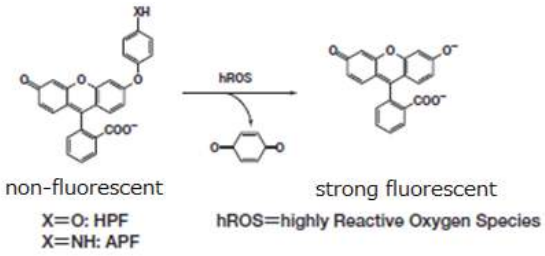
- **HFP and AFP detect selectively highly reactive oxygen species (hROS) such as hydroxyl radical ($^{\cdot}OH$) and peroxynitrite ($ONOO^-$), whereas they do not react with other reactive oxygen species ($O^{\cdot-}_2$, H_2O_2, $^1O_2$, NO, etc.).**
- **Quantitative analysis of H_2O_2 is available by adding HRP.**
- **Detecting specifically hypochlorite (OCl^-) by the combination use of HPF and APF.**
- **HPF and APF are not autoxidized at all, which enables us to easily get more reliable data than by other reagents.**
- **Live fluorescence imaging of cells is available.**

Principle of the measurement

HPF and APF are almost non-fluorescent in neutral solution. When they react with highly reactive oxygen species (hROS), they generate fluorescein that has high fluorescence intensity (excitation wave length: 490 nm, fluorescent wave length: 515 nm), and increase in the intensity of the fluorescence is observed.

Principle of the measurement

1mg of HPF / APF is dissolve in N,N -dimethylformamide (DMF) 0.47 mL. Their concentration are 5 mmol/L, each. Dilute the reagent 500~5000 times (10~1μmol/L) by Phosphate buffer (0.1 mol/L, pH 7.4) before use. Adding the diluted reagent to the sample and incubate, then measure the fluorescence of wave length: 515 nm with excitation of wave length: 490 nm.

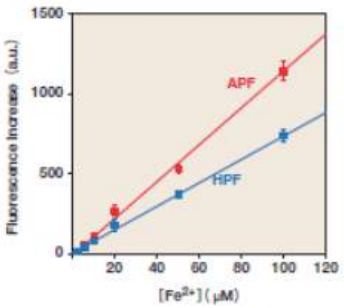


Fluorescent probes for detecting reactive oxygen species (ROS)

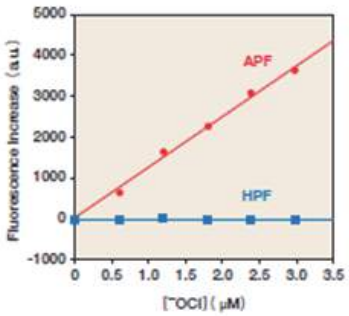
Each fluorescent reagent (final concentration: 10 mM, added 0.1 % DMF as co-solvent) was reacted with various reactive oxygen species in phosphate buffer (0.1 M, pH 7.4).

Fluorescent intensity of HPF, APF and DCFH was measured at 515, 515, 520nm by the excitation of 490, 490, 500 nm, respectively.

ROS	HPF	APF	DCFH
$^{\cdot}OH^a$	730	1200	7400
$ONOO^{-b}$	120	560	6600
$^{-}OCl^c$	6	3600	86
$^1O_2{}^d$	5	9	26
$O_2^{\cdot-e}$	8	6	67
$H_2O_2{}^f$	2	<1	190
NO^g	6	<1	150
$ROO^{\cdot h}$	17	2	710
Autoxidation^i	<1	<1	2000



Detection of $^{\cdot}OH$



Detection of OCl^-

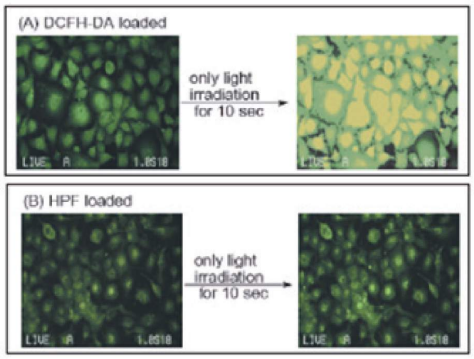
Application 1 Light-induced autoxidation in cells

Adding fluorescent probe

Incubate the cells for 30min at 37 ℃ in the dark with HPF or DCFH-DA (10 mM each).

Measuring fluorescence

Observe by the confocal scanning microscope (with excitation of 488 nm, and observing fluorescence of over 515 nm).
Irradiate laser with wave length of 488 nm to the cells for 10 s, and then observe again.



Application 2 Bioimaging of porcine neutrophils

Purification of porcine neutrophil

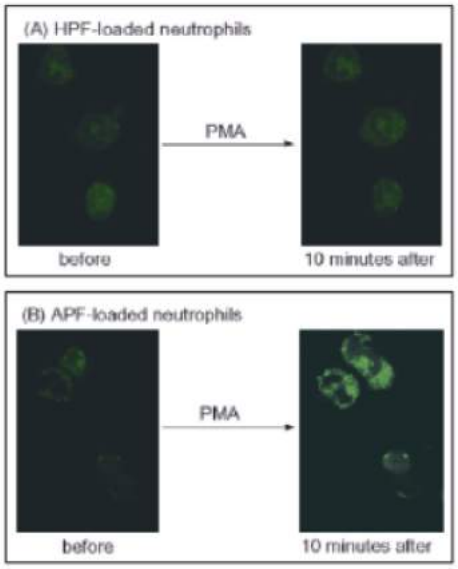
Isolate neutrophils from porcine blood.
Dissolve the purified neutrophils in Krebs-Ringer phosphate buffer (114 mM NaCl, 4.6 mM KCl, 2.4 mM $MgSO_4$, 1.0 mM $CaCl_2$, 15 mM NaH_2PO_4/Na_2HPO_4, pH 7.4) and incubate them on ice till before use.
Then, move them on the dish.

Adding probe and Stimulating by PMA

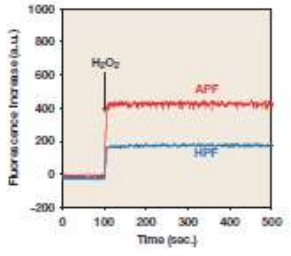
Add 10 mM of HPF or APF and incubate for 30 min at room templature.
Stimulate the neutrophils by adding 4 β-phorbol-12-myristate-13-acetate（PMA）（2 ng/mL, with 0.1 % DMF as co-solvent).

In vivo imaging of neutrophils

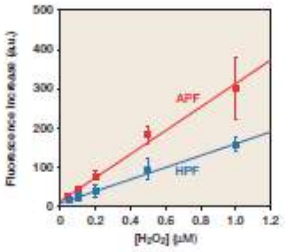
Obsrerve the neutrophils by the confocal scanning microscope (with excitation of 488 nm, and filter of 505-550 nm for the observation) before and 10 min after the stimulation.



Application 3 Detection of hROS in the HRP/H_2O_2 system using HPF and APF



Add 1μM of H_2O_2 to the each HPF/APF fluorescent reagent (final concentration: 10 mM, added 0.1 % DMF as co-solvent) in phosphate buffer (0.1 M, pH 7.4) containing 0.2 μ M HRP.
Measure the intensity of fluorescence of HPF or APF at 515 nm with excitation of 490 nm.



Increase the density of H_2O_2 up to 1μM.
Measure the intensity of fluorescence of HPF or APF at 515 nm with excitation of 490 nm.

Code No.	Product	Size	Storage
SK3001-01	Hydroxyphenyl Fluorescein (HPF) [1)]	1mg	2~10℃
SK3002-01	Aminophenyl Fluorescein (APF) [1)]	1mg	2~10℃

[1)] Dissolved in DMF

Reference

1. Setsukinai K., Urano Y., Kakinuma K., Majima H.J. and Nagano T.(2003)J. Biol. Chem . 278, 3170-3175 Cell. Biol. 2002, 158, 215-220

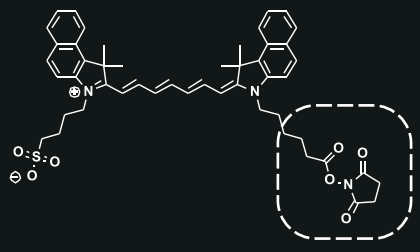
- Labeling with NHS esters, maleimides or through click reaction.
- Labeling kits are available.
- 【Custom labeling service】Synthesized in our lab in Japan.

Best probe for in vivo near-infrared fluorescence imaging

ICG Near Infrared Fluorescent Dyes

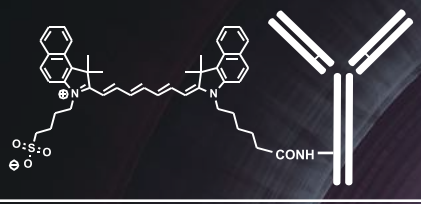
Line-up of products

We have a large variety of ICG near infrared fluorescent probes for labeling various molecules or antibodies in order to meet the needs of scientists.

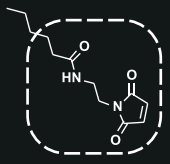


NHS series
Labeling antibody by amide bond.

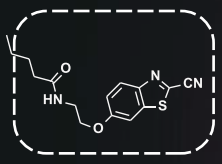
Reacting site



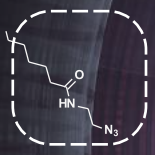
Labeling antibody by amide bond.



Maleimide series
Labeling cysteine residues.



CBT series
Labeling cysteine residues.



Azide series
Labeling easily by Click reaction

Custom ICG labeling service

We can conjugate your antibody with ICG. Please download application documents, fill them out and send it to us by email. Please ask us about price or delivery schedule.

NHS series (Most commonly used method for labeling)

Labeling of antibodies by amide bonding. Labeling kit is available.

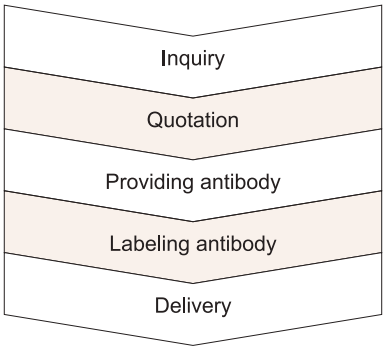
Code No.	Product	Size	Remarks
IM104	ICG NHS ester (high purity)	1mg	For in vivo fluorescent imaging. Labeling of antibodies by amide bonding. Excitation/Emission maximum (nm): 790/830
IM105	ICG NHS ester (high purity)	5mg	For in vivo fluorescent imaging. Labeling of antibodies by amide bonding. Excitation/Emission maximum (nm): 790/830
IM106	ICG NHS ester (high purity)	10mg	For in vivo fluorescent imaging. Labeling of antibodies by amide bonding. Excitation/Emission maximum (nm): 790/830

Maleimide series (Labeling the thiol groups of cysteine residues)

Labeling the thiol groups of cysteine residues. All you have to do for the labeling is just mixing. Labeling kit is available.

Code No.			
IM107	ICG maleimide	1mg	Labeling thiols of proteins with maleimide.
IM108	ICG maleimide	5mg	Labeling thiols of proteins with maleimide.
IM109	ICG maleimide	10mg	Labeling thiols of proteins with maleimide.

Flow Chart of contract ICG-antibody labeling service



Procedure for ordering
Please ask us to inquire about price and scheduling.

Filling out the application documents and sending them
Please download all application documents and fill out them.
Send us the filled-out documents before send us your sample.

Sending your sample
Send us your sample and application documents.
Be sure to pack the sample carefully to avoid drying, leakage or corruption of the vial.
The sample should reach us weekday morning.

Deliverables
Work report and final products of contracted work, such as purified antibodies.

Indocyanine Green Labeling Kit

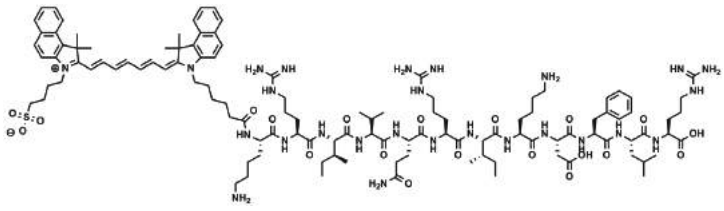
Indocyanine Green-NHS (succinimidyl ester) is near-infrared fluorescent probe that binds to amino acid residues without any condensing agents. Not only proteins or antibodies but also oligo-nucleotide with amino acid at the end can be labeled. This kit contains reagents and tools that required for the labeling for five times with Indocyanine Green-NHS and purification. One package of Indocyanine Green-NHS is optimum for the labeling of 300mg of proteins, such as IgG.

【labeling kit】

- Indocyanine Green-NHS ×5
- Dimethylsulfoxide 500μL ×1
- Reaction Buffer 5mL ×1
- Washing Buffer 10mL ×1
- Spin column for ultrafiltration ×5

Code No.	Product	Labeling method	Size
IM118	Indocyanine Green Labeling Kit	NHS-ester	For 5 times
IM134	Indocyanine Green Labeling Kit	NHS-ester	For 1 times

An example of ICG labeling
(Labeling of antimicrobial peptide with NHS)



Reference

1. Boudewijn E. Schaafsma, MD, J.Sven D. Mieog, MD, Merlijn Hutteman, MSc, Joost R. van der Vorst, MD, Peter J.K. Kuppen, PhD, Clemens W.G.M. Löwik, PhD, John V. Frangioni, MD, PhD, Cornelis J.H. van de Velde, MD, PhD, and Alexander L. Vahrmeijer, MD, PhD,* J Surg Oncol. 2011 Sep 1; 104(3): 323–332., "The clinical use of indocyanine green as a near-infrared fluorescent contrast agent for image-guided oncologic surgery"

Functionalized Near Infrared Fluorescent Dyes
For in vivo imaging using near infrared fluorescent dyes

ICG Line-up

Line-up of ICG near infrared fluorescent dyes

Code No.	Product	Size	Remarks
IM101	ICG NHS ester	1mg	For in vivo fluorescent imaging. Labeling of antibodies by amide bonding. Excitation/Emission maximum (nm): 790/830
IM102	ICG NHS ester	5mg	For in vivo fluorescent imaging. Labeling of antibodies by amide bonding. Excitation/Emission maximum (nm): 790/830
IM103	ICG NHS ester	10mg	For in vivo fluorescent imaging. Labeling of antibodies by amide bonding. Excitation/Emission maximum (nm): 790/830
IM104	ICG NHS ester (high purity)	1mg	For in vivo fluorescent imaging. Labeling of antibodies by amide bonding. Excitation/Emission maximum (nm): 790/830
IM105	ICG NHS ester (high purity)	5mg	For in vivo fluorescent imaging. Labeling of antibodies by amide bonding. Excitation/Emission maximum (nm): 790/830
IM106	ICG NHS ester (high purity)	10mg	For in vivo fluorescent imaging. Labeling of antibodies by amide bonding. Excitation/Emission maximum (nm): 790/830
IM107	ICG maleimide	1mg	Modify to the thiol groups in protein by maleimide.
IM108	ICG maleimide	5mg	Modify to the thiol groups in protein by maleimide.
IM109	ICG maleimide	10mg	Modify to the thiol groups in protein by maleimide.
IM110	ICG CBT	1mg	Labeling selectively cysteine residues in proteins or antibodies by cyanobenzothiazlole groups.
IM111	ICG CBT	5mg	Labeling selectively cysteine residues in proteins or antibodies by cyanobenzothiazlole groups.
IM112	ICG CBT	10mg	Labeling selectively cysteine residues in proteins or antibodies by cyanobenzothiazlole groups.
IM113	ICG azide	1mg	Labeling protein and antibody by Azide-Alkyne cycloaddition (Click reaction)
IM114	ICG azide	5mg	Labeling protein and antibody by Azide-Alkyne cycloaddition (Click reaction)
IM115	ICG azide	10mg	Labeling protein and antibody by Azide-Alkyne cycloaddition (Click reaction)
IM116	ICG carboxylic acid	—	ICG with carboxyl group. For custom synthesis of labeling.
IM117	ICG PEG NHS ester	—	ICG with water soluble linker.
IM118	Indocyanine Green Labeling Kit	5times	ICG antibody labeling kit

Code No.	Product	Size	Remarks
IM119	IR-820 NHS ester	1mg	For in vivo fluorescent imaging. Labeling protein and antibodies by amide bonding. Excitation/Emission (nm): 710/820
IM120	IR-820 NHS ester	5mg	For in vivo fluorescent imaging. Labeling protein and antibodies by amide bonding. Excitation/Emission (nm): 710/820
IM121	IR-820 NHS ester	10mg	For in vivo fluorescent imaging. Labeling protein and antibodies by amide bonding. Excitation/Emission (nm): 710/820
IM122	IR-820 maleimide	1mg	Modify to the thiol groups in protein by maleimide.
IM123	IR-820 maleimide	5mg	Modify to the thiol groups in protein by maleimide.
IM124	IR-820 maleimide	10mg	Modify to the thiol groups in protein by maleimide.
IM125	IR-820 CBT	1mg	Labeling selectively cysteine residues in proteins or antibodies by cyanobenzothiazlole groups.
IM126	IR-820 CBT	5mg	Labeling selectively cysteine residues in proteins or antibodies by cyanobenzothiazlole groups.
IM127	IR-820 CBT	10mg	Labeling selectively cysteine residues in proteins or antibodies by cyanobenzothiazlole groups.
IM128	IR-820 azide	1mg	Labeling protein and antibody by Azide-Alkyne cycloaddition (Click reaction)
IM129	IR-820 azide	5mg	Labeling protein and antibody by Azide-Alkyne cycloaddition (Click reaction)
IM130	IR-820 azide	10mg	Labeling protein and antibody by Azide-Alkyne cycloaddition (Click reaction)
IM131	IR-820 alkyne	1mg	Labeling protein and antibody by Azide-Alkyne cycloaddition (Click reaction)
IM132	IR-820 alkyne	5mg	Labeling protein and antibody by Azide-Alkyne cycloaddition (Click reaction)
IM133	IR-820 alkyne	10mg	Labeling protein and antibody by Azide-Alkyne cycloaddition (Click reaction)

A series of fluorescent dyes originally released from Goryo Chemical Inc.

STELLAFluor™ Series

Goryo Chemical Inc. provides you a series of fluorescent dyes, STELLA Fluor™ Series.
Japanese special manufacturer of fluorescent chemicals guarantee the quality of our products.
We manage wide range of fluorescent wave length. Feel free to ask us.

This series of fluorescent dye optimizes generally-used dyes for labeling. We support various labeling sites depending on the labeling molecules and methods, similarly to POLARIC™-labeling series. Please choose appropriate dyes depending on your use. We receive your target protein and label it with the florescent dye that you want.

Product		Size	Code No.	Corresponding representative dye
STELLA Fluor™ 488	NHS	1mg	ST1003-11	Calcein, Rhodamine123 FITC BODIPY® 492/515 Alexa Fluor®488
		5mg	ST1003-15	
	maleimide	1mg	ST1003-21	
		5mg	ST1003-25	
	free COOH	1mg	ST1003-31	
		5mg	ST1003-35	
	free NH_2	1mg	ST1003-41	
		5mg	ST1003-45	
STELLA Fluor™ 600	NHS	1mg	ST1006-11	Cy™3 Texas Red DsRed BODIPY® 564/570
		5mg	ST1006-15	
	maleimide	1mg	ST1006-21	
		5mg	ST1006-25	
	free COOH	1mg	ST1006-31	
		5mg	ST1006-35	
	free NH_2	1mg	ST1006-41	
		5mg	ST1006-45	
STELLA Fluor™ 650	NHS	1mg	ST1008-11	Alexa Fluor® 647 ATTO® 647N Cy™5 DyLight™ 649 Qdot® 655
		5mg	ST1008-15	
	maleimide	1mg	ST1008-21	
		5mg	ST1008-25	
	free COOH	1mg	ST1008-31	
		5mg	ST1008-35	
	free NH_2	1mg	ST1008-41	
		5mg	ST1008-45	
STELLA Fluor™ 700	NHS	1mg	ST1010-11	Alexa Fluor® 700
		5mg	ST1010-15	
	maleimide	1mg	ST1010-21	
		5mg	ST1010-25	
	free COOH	1mg	ST1010-31	
		5mg	ST1010-35	
	free NH_2	1mg	ST1010-41	
		5mg	ST1010-45	
STELLA Fluor™ 720	NHS	1mg	ST1011-11	Cy™7 DY-730
		5mg	ST1011-15	
	maleimide	1mg	ST1011-21	
		5mg	ST1011-25	
	free COOH	1mg	ST1011-31	
		5mg	ST1011-35	
	free NH_2	1mg	ST1011-41	
		5mg	ST1011-45	

Bioluminescent luciferin substrates and other probes for in vivo imaging

Bioluminescent Series

Code No.	Product	Size	Remarks
IM201	D-Luciferin free acid (Standard Purity)	1g	For in vivo and in vitro assay using bioluminescence.
IM202	D-Luciferin free acid (Standard Purity)	5g	For in vivo and in vitro assay using bioluminescence.
IM203	D-Luciferin potassium salt (Standard Purity)	1g	Higher water-solubility
IM204	D-Luciferin potassium salt (Standard Purity)	5g	Higher water-solubility
IM205	D-Luciferin free acid (High Purity)	1g	For in vivo and in vitro assay using bioluminescence.
IM206	D-Luciferin free acid (High Purity)	5g	For in vivo and in vitro assay using bioluminescence.
IM207	D-Luciferin potassium salt (High Purity)	1g	Higher water-solubility
IM208	D-Luciferin potassium salt (High Purity)	5g	Higher water-solubility
IM209	Slow release D-luciferin (split luciferin) cell assay kit	—	Producing a luciferin in vivo. It has high chemical stability, high cell permeability, and stable signal can be obtained.
IM210	Caspase 3 and Caspase 7 5-mouse kit (z-DEVD-D-Cys)	—	For in vivo caspase activity
IM211	Caspase 8 5-mouse kit (z-IETD-D-Cys)	—	For in vivo caspase activity
IM212	6-Hydroxy-2-cyanobenzothiazole (hydroxy-CBT)	50mg	D-Luciferin precursor
IM213	6-Hydroxy-2-cyanobenzothiazole (hydroxy-CBT)	100mg	D-Luciferin precursor
IM214	6-Amino-2-cyanobenzothiazole (amino-CBT)	50mg	D-Aminoluciferin precursor
IM215	6-Amino-2-cyanobenzothiazole (hydroxy-CBT)	100mg	D-Aminoluciferin precursor
IM216	Fatty acid uptake probe	0.5mg	Fatty acid uptake probe
IM217	Fatty acid uptake probe	1mg	Fatty acid uptake probe
IM218	Fatty acid uptake probe	5mg	Fatty acid uptake probe
IM219	BacteriTrace Vanco	0.5mg	For in vivo imaging of bacterial infection.
IM220	BacteriTrace Vanco	1mg	For in vivo imaging of bacterial infection.
IM221	BacteriTrace Vanco	5mg	For in vivo imaging of bacterial infection.

Fluorescent dye for cell staining

POLARIC™ Series

POLARIC™ -500c6F

Fluorescent dye for cell staining

POLARIC™-500c6F is solvatochromic dye developed for cell staining. Solvatochromic dye means the dyes changing fluorescent colors by environment such as localizing to organelle, etc.
POLARIC™-500c6F is most appropriate for time-lapse observation because it is held in cell long time, compere to other dyes.

- **Staining both mitochondria and cell membrane by the single dye**
- **Superior retentivity in cells**
- **Low toxicity**

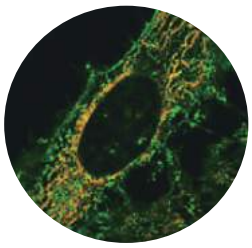


Fig. 1. Image of cell membrane and mitochondria stained by POLARIC™-500c6F.

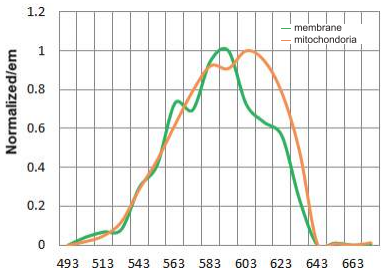


Fig. 2. Fluorescent spectra of plasma membrane and mitochondria stained by POLARIC™-500c6F.
Spectral data were acquired from the image of HeLa cell obtained by confocal laser-scanning microscopy. Spectra changed depending on the environment around the dye, at plasma membrane and mitochondria.

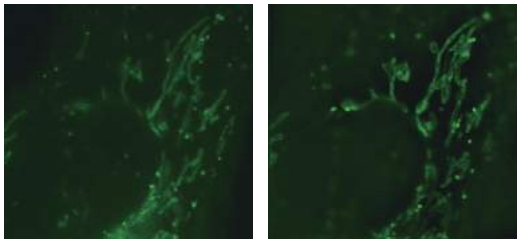


Fig. 3. Images of HeLa cell obtained by Structured Illumination Microscope (Nikon, N-SIM) after staining by POLARIC™-500c6F. Left: Image taken by conventional epi-fluorescent microscopy. Right: Image taken by 3D-SIM. Ex: 488 nm, Em: 510-550 nm.
Image obtained by SIM, whose spatial resolution is about twice (about 100nm) of conventional microscopy, showed the detailed structure of mitochondria.

Code No.	Product	Size	Remarks
GC101	POLARIC™-500c6F	1μg × 10	For live cell imaging.
GC1011	POLARIC™-500c6F	1μg × 5	For live cell imaging. (Small size for trial)
GC102	POLARIC™-500BCS	5μg × 10	For imaging of bacteria.
GC1021	POLARIC™-500BCS	5μg × 5	For imaging of bacteria. (Small size for trial)
GC211	POLARIC™ Labeling Kit	For 5 times	A value all-in-one kit for labeling.
GC2011	POLARIC™-ITC	1 mg	For fluorescent modification with isothiocyanate.
GC2021	POLARIC™-NHS	1 mg	For fluorescent labeling by amide bonding.
GC2031	POLARIC™-MLI	1 mg	For fluorescent labeling thiols with maleimide.
GC2041	POLARIC™-COOH	1 mg	For fluorescent labeling using cross linker.
GC2051	POLARIC™-NH2	1 mg	For fluorescent labeling using cross linker.
GC2061	POLARIC™-BIO	1 mg	Biotin conjugated.
GC2071	POLARIC™-AVI	1 mg	Avidin conjugated.
GC2081	POLARIC™-STA	1 mg	Streptavidin conjugated.

References

1. Chem. Lett. 2011, 40, 989. Direct detection of ABCA1-dependent HDL formation based on lipidation-induced hydrophobicity change in apoA-I.
2. Omura R, Nagao K, Kobayashi N, Ueda K, Saito H., J Lipid Res. 2014 Nov;55(11):2423-31, "Direct detection of ABCA1-dependent HDL formation based on lipidation-induced hydrophobicity change in apoA-I."
3. Maishi N, Kawamoto T, Ohga N, Yamada K, Akiyama K, Yamamoto K, Osawa T, Hida Y, Hida K., Oncol Rep. 2013 Oct;30(4):1695-700., "Application of POLARIC™ fluorophores in an in vivo tumor model."
4. Osakai T, Yoshimura T, Kaneko D, Nagatani H, Son SH, Yamagishi Y, Yamada K, Anal Bioanal Chem. 2012 Aug;404(3):785-92., "Potential-modulated fluorescence spectroscopy of zwitterionic and dicationic membrane-potential-sensitive dyes at the 1,2-dichloroethane/water interface."

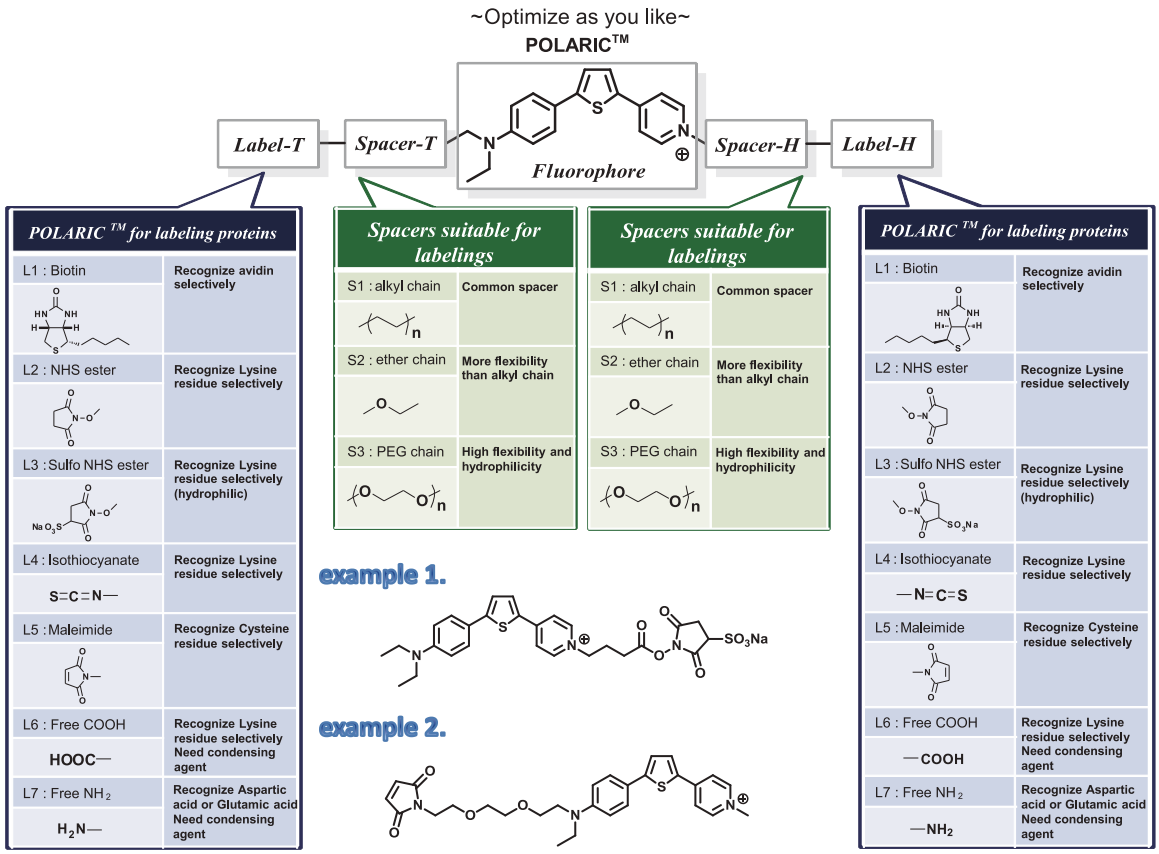
Custom Services

Labeling Services

POLARIC™ Labeling services

POLARIC™ Protein and Antibody labeling

we can conjugate your nucleotide, molecular target drug candidate matter, antibody and protein to POLARIC™ labels.



example 1.

example 2.

Custom Labeling Service

Workflow from ordering to reception.		Documents
Inquiry	Please contact us from “Contact us” on HP, Email(info@polaris-t.com) or tel(+81-11-214-9422)	
Estimate	We make the (approximate) estimate on the basis of hearing. (sine non-disclosure agreement if needed)	Estimate (NDA)
Business trust agreement	Entering into Business trust agreement.	Business trust agreement
Receive Samples	Please send us your samples and information after agreement.	
Labeling	We produce Fluorscent labeling material according to experimental plan and report on progress if you needed.	Progress report
Completion and Delivery	We send to you Fluorescent labeled material.	Packing list
Experimental Report	After the end of operation, we submit experimental report.	Experimental Report
Charge	We send you invoice. We talk about the terms of payment separately.	invoice

Change or abbreviation of agreement workflow available.

Custom Services

Labeling Services

Other Fluorophores Labeling services

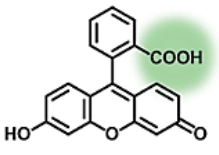
Other Fluorophores Protein and Antibody labeling

Optimization of Fluorescent Labeling

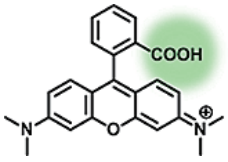
Custom Fluorescent labeling service is common Fluorescent dye labeling service.
We provide various common dyes what have labeling site according to labeled molecules and method.
Please choose the best dye.
We take your nucleotide, protein or antibody and we conjugate it.

examples of Fluorescent dyes

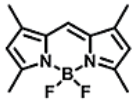
Non-fluorescent TokyoGreen®-βGal is taken into the cell, is hydrolyzed by the β-galactosidase, and generates bright fluorescent TokyoGreen®.
TokyoGreen® is also permeable through the cell membrane, so that generated TokyoGreen® diffuses uniformly in the culture medium and that the whole medium makes green fluorescence (510 nm) when it is irradiated by the 490 nm excitation light.



Fluorescein



Rhodamine



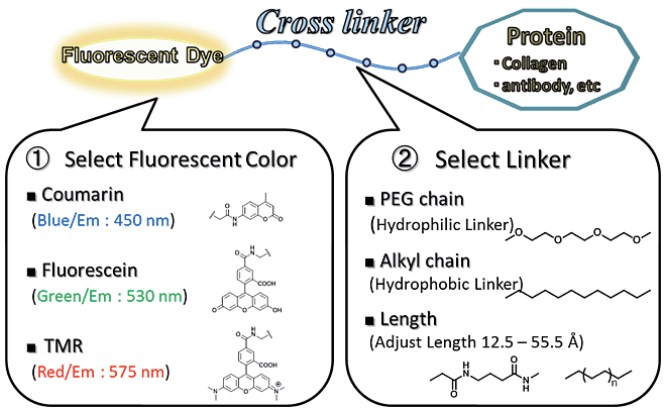
Boron dipyrromethene

The list of common fluorescent dye (selection)

Color	Fluorescnt wavelength Representative dyes	Examples of dyes we can conjugate	Representative dyes
Blue	400-500nm	Coumarin	DAPI Hoechst33342 Propidium Iodide SYTOX®Blue Pacific BlueTM Alexa Fluor®430
Green	500-550nm	Fluorescein, Boron dipyrromethene	GFP PE Calcein, Rhodamine123 FITC BODIPY 492/515 Alexa Fluor®488
Red	550-650nm	Rhodamine, Boron dipyrromethene	Cy™3 Texas Red DsRed BODIPY 564/570 Alexa Fluor®555 Rhodamine Phalloidin
IR-Red	650nm-	Cyanine	Cy™5 Cy™7 BODIPY 650/665 Dye Alexa Fluor®647

Sytox®, Pacific Blue™ and AlexaFluor® are registered trademarks of LifeTechnologies Company.
Cy™ are registered trademarks of GE Healthcare Company.

Optimization of Fluorescent dye



Product Guarantee

Elimination of antibody-activity do not guarantee due to Fluorescent labeling. Please be forewarned.

Custom Antibody Labeling Service

Outline

Provide us your Antibody	Please provide us purified antibody (IgG purification, Affinity purification)
Pilot Test	We conjugate a part of your antibody.
Confirm	Please confirm activity of antibody.
Conjugate all	We conjugate all of your antibody.
Delivery	We delivery labeled antibody with report.

Custom Labeling Service

Workflow from ordering to reception.

		Documents
Inquiry	Please contact us from “Contact us” on HP, Email(info@polaris-t.com) or tel(+81-11-214-9422)	
Estimate	We make the (approximate) estimate on the basis of hearing. (sine non-disclosure agreement if needed)	Estimate (NDA)
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Experimental Report	after the end of operation, we submit experimental report.	
Charge	We send you invoice. We talk about the terms of payment separately.	invoice

Change or abbreviation of agreement workflow available.

Term	Needed amount of antibody	Delivery Time
Fluorescent labeling	5-20mg	About 4 weeks

Order Made Services

Labeling Services

Contract Uptake Assay Systems of Fluorescent-Labeled Substances

- **Quantitating the activity of uptaking of substance by cells using fluorescence**
- **Easy to screen many samples by 96well plate**

We label the substance with fluorescence and produce your own assay plate. Drugs which enhance/atteuate activity of uptake of substance by cells are quantified simply. We label your substance with fluorescence your request.

Example of exam Quantitation uptaking of TAMRA labeled amyloid-β by Microglia cell

<Method>

1. Label amyloid-β with TAMRA by solid-phase synthesis.
2. Oligomerize TAMRA labeled amyloid-β and coated on 96 well plate.
3. Seed microglia cell.
4. Incubate for 24 hours, collect culture supernatant and measure fluorescence intensity(Ex.:555nm, Em.:585nm). Control experiment is performed in the well with no cell.

Results

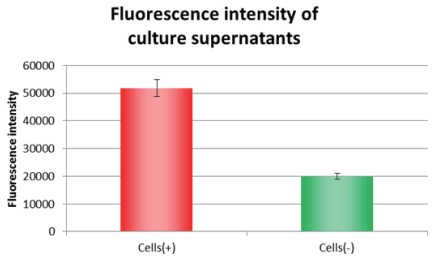


Fig.1. TAMRA labeled amyloid-β oligomer were incorporated by Microglia cell and after 24 hours, collected supernatants were measured fluorescence intensity. It is suggested that microglia cells incorporated TAMRA labeled amyloid-β and released to culture supernatant.

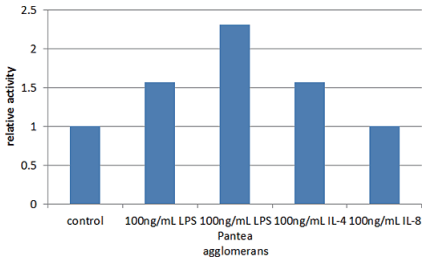
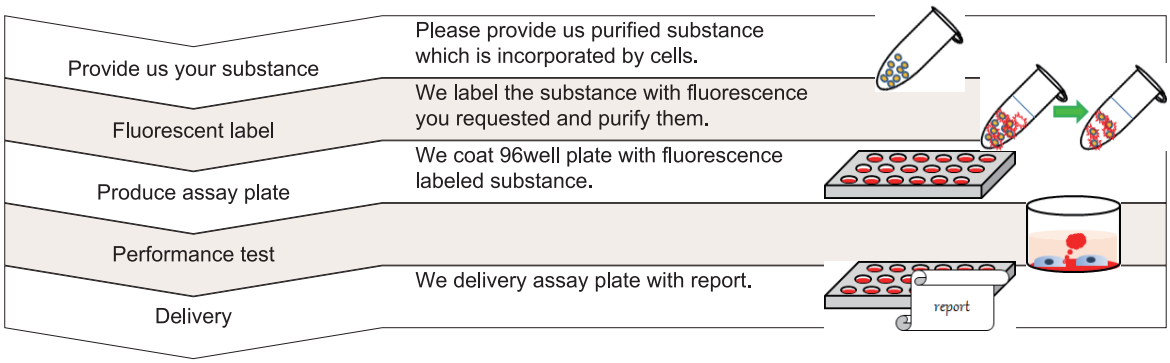


Fig.2. Microglial cells was seeded on the plate which was coated with TMR-βamyloid oligomer and stimulated with various drugs. After 24 hours, the supernatant was collected and fluorescence intensity was measured. The Graph indicates relative change of uptake activity (additive-free Control is assumed 1) of TMR-β amyloid oligomer by each drugs.

Goryo Chemical, Inc. Custom Assay System survice

Outline



Term	Unit	Delivery Time
Fluorescence labeled assay plate	1plate	About 4 weeks

Coming soon!

- Fluorescent probes detecting specific ROS species
- Florescent probe detecting copper ion
- MitoAR/MitoHR
- Photo-sensitizer localizing to specific cells

GORYO CHEMICAL

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