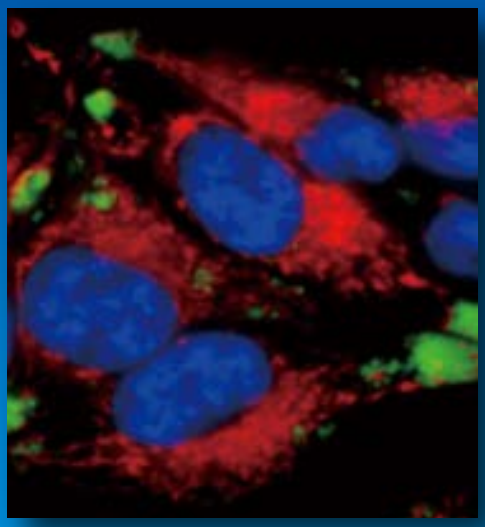
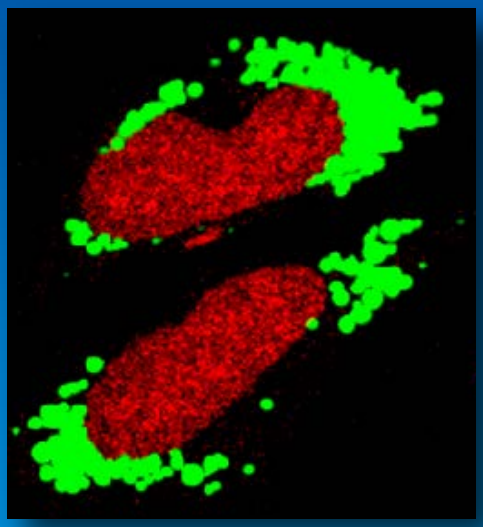
LIFE SCIENCE

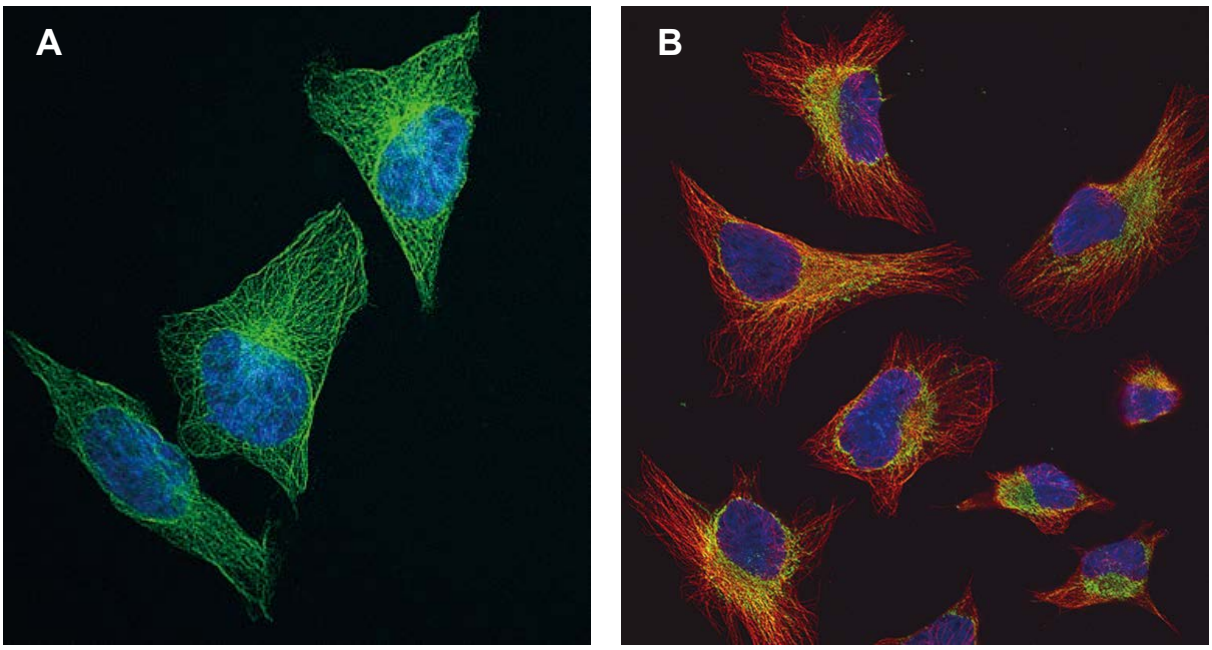
Cell Imaging Reagents





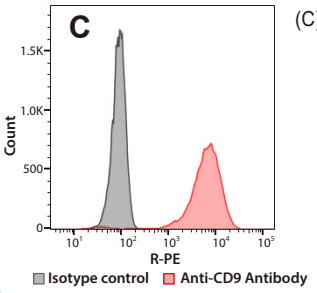
Fluorescent Labeled Secondary Antibodies and Fluorescent Cell Stains

Applications



(A) The HeLa cells were incubated with properly diluted primary antibody (Mouse Anti α-Tubulin IgG) and were further incubated with Goat Anti-Mouse IgG Biotin Conjugate [G0387] and Streptavidin FITC Conjugate [S0966] (green fluorescence). And then the nuclei was stained with DAPI 2HCl [A2412] (blue fluorescence). (Laser Scanning Microscope: Olympus FLUOVIEW FV 3000)

(B) The nuclei of HeLa cells was stained with Bisbenzimide H 33258 [H1343] (blue fluorescence). α-Tubulin was stained with anti-α-tubulin antibody and Goat Anti-Mouse IgG Biotin Conjugate [G0387] and Streptavidin R-PE Conjugate [T3885] (red fluorescence). Mitochondria was stained with primary antibody and Goat Anti-Rabbit IgG FITC Conjugate [G0452] (green fluorescence)**. (Laser Scanning Microscope: Olympus FLUOVIEW FV 3000)



(C) The HeLa cells were incubated with Mouse Anti-CD9 Antibody (red line) or Mouse IgG2aκ isotype control (black line). Subsequently, both were stained with Goat Anti-Mouse IgG Biotin Conjugate [G0387] and Streptavidin R-PE Conjugate [T3885]. (Flow cytometer: Sysmex RF-500)

Since these products are comprised of premade solution,

**Please refer to our product page for staining procedure.
R-PE/FITC-labeled anti-Mouse IgG or anti-Rabbit IgG antibodies and streptavidins can be used for fluorescence immunostaining and flow cytometry.

Goat Anti-Mouse IgG FITC Conjugate	(Green Fluorescence)	0.1mg/vial	[G0406]
Goat Anti-Mouse IgM FITC Conjugate	(Green Fluorescence)	0.1mg/vial	[G0453]
Goat Anti-Rabbit IgG FITC Conjugate	(Green Fluorescence)	0.1mg/vial	[G0452]
Streptavidin FITC Conjugate	(Green Fluorescence)	0.1mg/vial	[S0966]
Goat Anti-Mouse IgG R-PE Conjugate	(Red Fluorescence)	0.1mg/vial	[G0569]
Goat Anti-Mouse IgG_1 Fab Fragment Cyanine 3 Conjugate	(Red Fluorescence)	0.05mg/vial	[G0598]
Goat Anti-Rabbit IgG R-PE Conjugate	(Red Fluorescence)	0.1mg/vial	[G0577]
Streptavidin R-PE Conjugate	(Red Fluorescence)	0.1mg/vial	[T3885]
Goat Anti-Mouse IgG DTBTA-Eu^{3+} Conjugate	(Red Fluorescence)	0.1mg/vial	[G0505]
Goat Anti-Rabbit IgG DTBTA-Eu^{3+} Conjugate	(Red Fluorescence)	0.1mg/vial	[G0506]
Streptavidin DTBTA-Eu^{3+} Conjugate	(Red Fluorescence)	0.1mg/vial	[S0993]
DAPI 2HCl	(Blue Fluorescence)	5mg	[A2412]
DAPI 2HCl (1mg/mL in Water)	(Blue Fluorescence)	0.2mL x 5vial	[D5888]
Bisbenzimide H 33258 Hydrate	(Blue Fluorescence)	25mg	[H1343]
Bisbenzimide H 33258 (1mg/mL in Water)	(Blue Fluorescence)	0.2mL x 5vial	[B6236]

*Some products are unavailable in the Americas and China.
*The high-sensitivity detection of DTBTA-Eu^{3+} labeled probes requires time-resolved fluorometry.

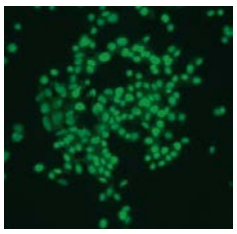
Please inquire for pricing and availability of listed products to our local sales representatives.

Cell Fluorescent Staining Dyes

Acridine Orange Solution [for Cell Staining] 5mL [A3396]

Application: The method of staining cells by A3396

1. Remove the medium from the culture plate and wash the cells twice with cold PBS(-). Remove the PBS(-).
2. Add PBS(-) and **A3396** (1/50th of the volume of the added PBS(-)) and incubate for 15 minutes.
3. Remove the staining solution and wash the cells twice with PBS(-).
4. Add PBS(-) and observe the cells under a fluorescence microscope.



NIH3T3 cells stained by **A3396**

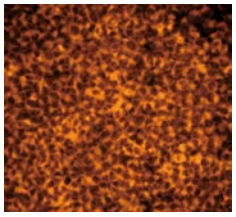
DiI Solution [for Cell Membrane Staining] 1mL [D6202]
DiD Solution [for Cell Membrane Staining] 1mL [D6263]

Advantages

- **Since these products are comprised of premade solution, simply add to culture medium to stain cell membranes.**
- **Once the hydrocarbon chains are incorporated into cell membranes, DiI emits orange fluorescence (Ex_{max}: 553 nm, Em_{max}: 575 nm) and DiD emits red fluorescence (Ex_{max}: 657 nm, Em_{max}: 678 nm) strongly.**

Application: Cell Membrane Staining by D6202

1. Culture cells and remove the medium.
2. Add medium supplemented with **D6202** to a final concentration of 5 μM, and incubate at 37°C for 10 minutes.
3. Remove the staining medium and wash three times with PBS(-).
4. Observe cell membranes using a fluorescence microscope.



HeLa cells stained by **D6202**

Chemiluminescence Reagent for the Detection of Superoxide

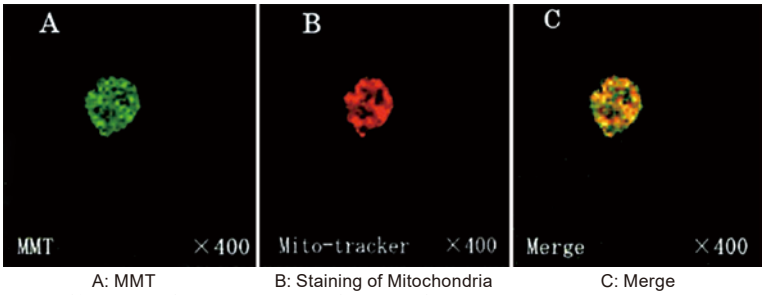
Lucigenin 1g / 5g [B1203]

MMT [= 10,10'-Dimethyl-9,9'-biacridinium Bis(monomethyl Terephthalate)] 100mg / 1g [B4339]

MMT (B4339) is a specific probe having lucigenin-like chemiluminescence to superoxide among reactive oxygen species. Since amphiphilic MMT which is less hydrophilic than lucigenin possesses cell-permeability, MMT has been applicable for the detection of intramitochondrial superoxide production.

Application

Figure shows the localization of MMT in mitochondria.



A: MMT (Green Fluor.) B: Staining of Mitochondria (Red Fluor.) C: Merge

Figure. Fluorescence in mitochondria in mouse peritoneal neutrophils. (Provided by Prof. Kobayashi)

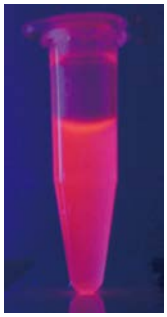
Reference S. Sasaki, Y. Kobayashi, *et al.*, *Free Radic. Biol. Med.* **2013**, *65*, 1005. https://doi.org/10.1016/j.freeradbiomed.2013.08.175

Europium Fluorescent Labeling Reagent

ATBTA-Eu^{3+} 10mg [A2083]

Cyanuric Chloride 25g / 500g [C0460]

ATBTA-Eu^{3+} is a europium chelate complex and can be used as a fluorescent labeling reagent.
ATBTA-Eu^{3+} is easily labeled to protein etc. after conversion to DTBTA-Eu^{3+} by Cyanuric Chloride.



Advantages

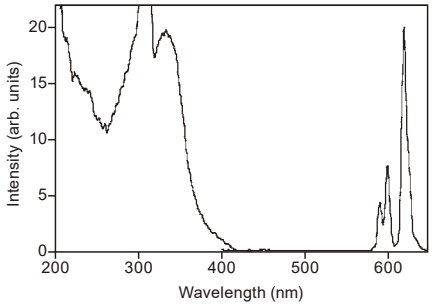
Long fluorescent life time (τ = 1.02ms*)
For time-resolved fluorometry

Stable fluorescence in various aqueous buffers
Available in Tris, TE PBS, etc. for wide use

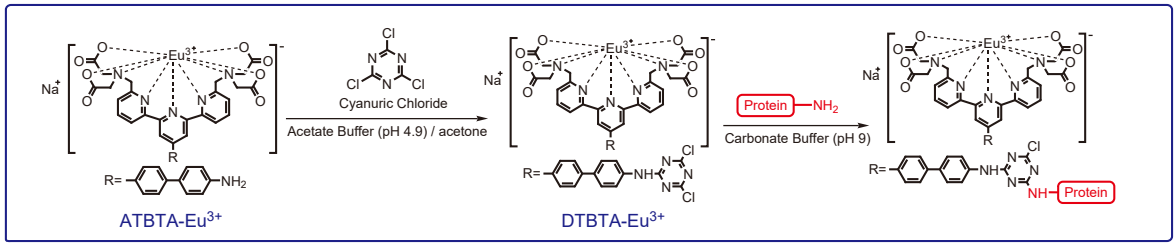
No cross talk of excitation light
- $\lambda_{ex,\,max}$ = 335nm*
- $\lambda_{em,\,max}$ = 616nm*

Sharpened emission spectrum Large Stokes shift (the difference in wavelength between positions of the band maxima of the absorption and emission spectra)

*Data as DTBTA-Eu^{3+}



Conversion of ATBTA-Eu^{3+} to DTBTA-Eu^{3+} and the labeling reaction to amino groups



■Typical Procedure (Preparation of DTBTA-Eu^{3+})

Dissolve 2 mg of ATBTA-Eu^{3+} in 60 µL of 0.1M acetate buffer (pH 4.9). This solution is added 0.43 mg of Cyanuric Chloride in 25 µL of acetone, and stirred for 30 minutes. The reaction mixture is added dropwise to 1 mL of acetone, and formed precipitate is centrifuged. After washing with 0.5 mL of acetone twice, the yellow powder is dried in vacuum for 1 hour. Dissolve the powder in 1 mL of carbonate buffer gives (pH 9) for labeling. This solution contains *ca.* 2 mM of labeling reagent.

■Warning

This labeling reagent is deactivated by hydrolysis, especially in alkali solution. The reagent dissolved in water should be used immediately. For temporary storage, the reagent should be dissolved in buffer solution at acidic pH (pH <5) and kept at 0 ºC.

DTBTA-Eu^{3+}-labeled Secondary Antibodies and Streptavidin

Goat Anti-Mouse IgG DTBTA-Eu^{3+} Conjugate 0.1mg/vial [G0505]
Goat Anti-Rabbit IgG DTBTA-Eu^{3+} Conjugate 0.1mg/vial [G0506]
Streptavidin DTBTA-Eu^{3+} Conjugate 0.1mg/vial [S0993]

Related Products (Anti-DTBTA-Eu^{3+} Antibody)

Anti-DTBTA-Eu^{3+} Rabbit Polyclonal Antibody 0.5mL [A2239]
Anti-DTBTA-Eu^{3+} Rabbit Antiserum 0.5mL [A2181]

† Some products are unavilable in the Americas and China.

Please inquire for pricing and availability of listed products to our local sales representatives.

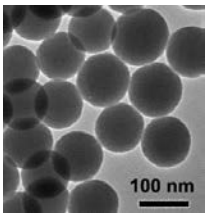
Fluorescent Organosilica Particles

Organosilica FITC (100nm Diam.) 2mg [O0561]

Organosilica Rhodamine B (100nm Diam.) 2mg [O0573]

O0561
Fluorescent image

O0573
Fluorescent image

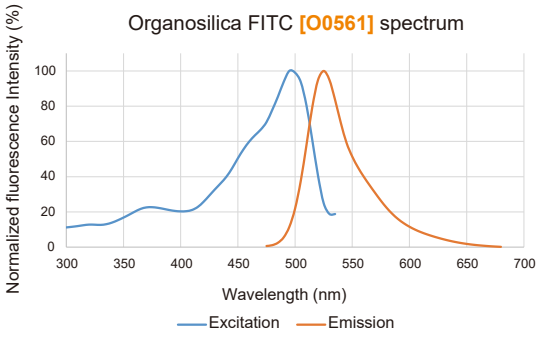


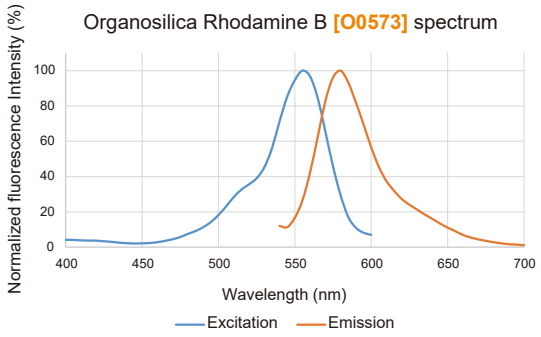
SEM image

Advantages

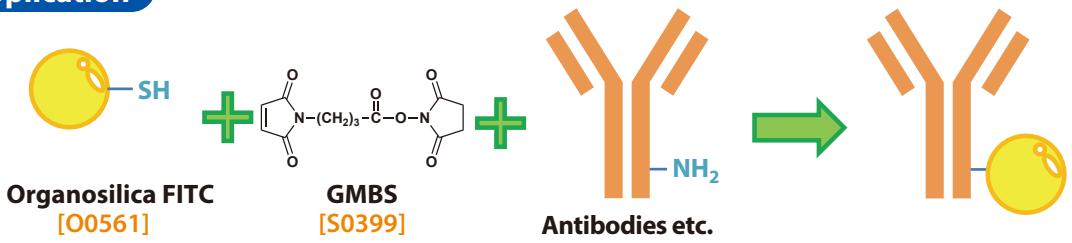
- Wavelength : Ex_{max} 492 nm, Em_{max} 523 nm (O0561)
  Ex_{max} 556 nm, Em_{max} 579 nm (O0573)
- Surface Functionalization : Thiol group (-SH)
- Superior in fluorescence intensity to the conventional FITC or rhodamine B.
- The diameter of these products are 100 nm and these products are suitable for the detection of biomolecules.

Fluorescence spectra





Application



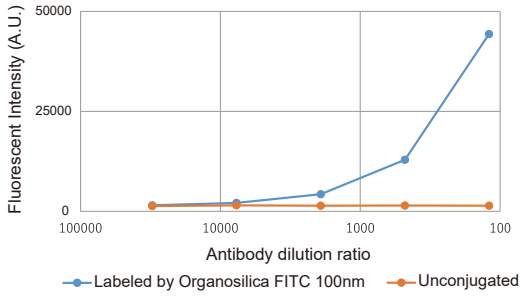
Organosilica FITC [O0561]

GMBS [S0399]

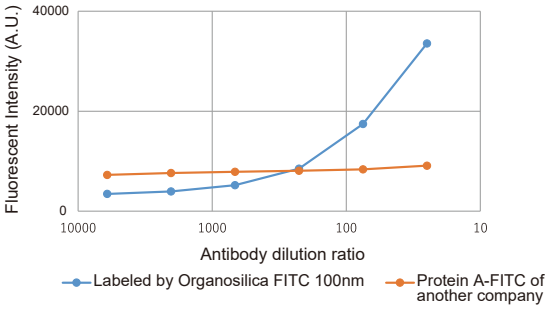
Antibodies etc.

Organosilica FITC [O0561] was labeled to various antibodies etc. by the above method. The fluorescence intensity of them at Ex: 485 nm, Em: 520 nm was measured.

Labeling with Goat anti Mouse IgG antibody



Labeling with Protein A



Organosilica FITC 100nm [O0561] could be labeled to various antibodies etc., and they were detected by fluorescence.

References M. Nakamura, *et al*., *ACS Nano* **2015**, *9*, 1058. https://doi.org/10.1021/nn502319r
M. Nakamura, *et al*., *Colloids Surf B Biointerfaces* **2010**, *79*, 19. https://doi.org/10.1016/j.colsurfb.2010.03.008

O0561 and O0573 are commercialized under the instruction of Prof. Michihiro Nakamura.

Lipid Droplet Fluorescent Staining Dyes

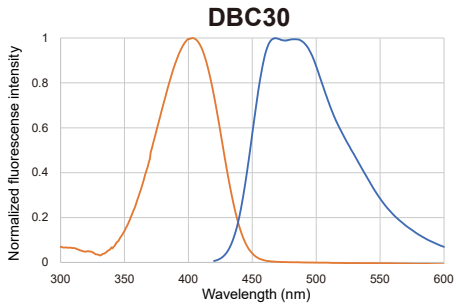
DBC30 (2mg/mL in Dimethyl Sulfoxide) [for Biochemical Research] 0.1mL [D6131]

PC6S (1mg/mL in Dimethyl Sulfoxide) [for Biochemical Research] 0.04mL / 0.2mL [P3152]

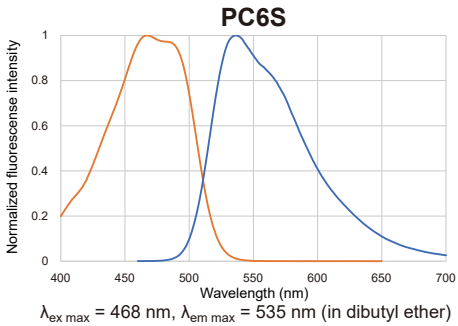
Advantages

- **Stains lipid droplets in both cells and tissues clearly and distinctly due to the high fluorescence intensity.**
- **Can be used in combination with multiple fluorescent dyes due to the high lipid droplet selectivity and intracellular retention.**

Excitation and Emission Spectra

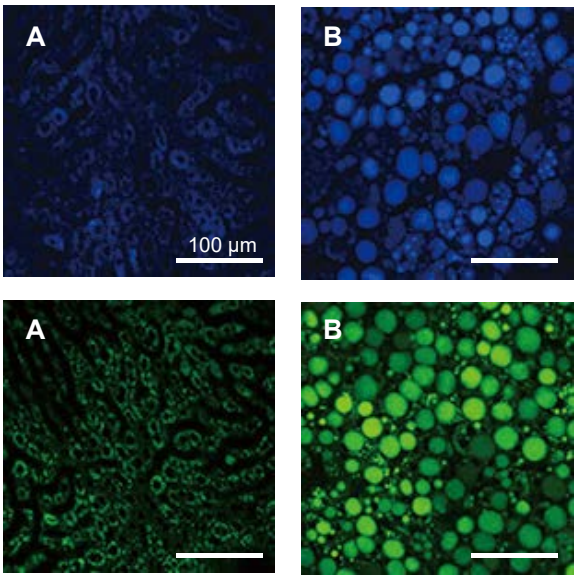


$\lambda_{ex\ max}$ = 403 nm, $\lambda_{em\ max}$ = 468 nm (in dibutyl ether)
Compatible with optical filter for DAPI.



$\lambda_{ex\ max}$ = 468 nm, $\lambda_{em\ max}$ = 535 nm (in dibutyl ether)
Compatible with optical filter for GFP or FITC.

Application : *in vivo* imaging of lipid droplets



The images of the surface of the mouse liver

Generation of fatty liver model mouse

- Feed Choline-deficient and L-Methionine-reduced ultra-high fat diet.

Lipid droplet imaging with DBC30 or PC6S

- Inject 50 nmol of DBC30 or PC6S into the tail vain of the mouse under anesthesia.
- After 30 minutes, expose the liver of the mouse and observe through confocal fluorescence microscope.

DBC30
Excitation wavelength : 405 nm
Observation wavelength : 440 - 480 nm

PC6S
Excitation wavelength : 488 nm
Observation wavelength : 500 - 540 nm

A : Healthy mouse
B : Fatty liver model mouse

These figures are provided by Dr. Toshitada Yoshihara.

References T. Yoshihara *et al.*, *Anal. Chem.* **2020**, *92*, 4996. https://doi.org/10.1021/acs.analchem.9b05184
K. Purevsuren *et al.*, *J. Photochem. Photobiol. A* **2023**, *438*, 114562. https://doi.org/10.1016/j.jphotochem.2023.114562

D6131 and P3152 are commercialized under the instruction of Dr. Toshitada Yoshihara.

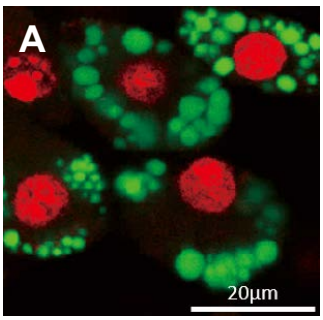
ShoyakuGreen (= TFMAQ-8Ph)

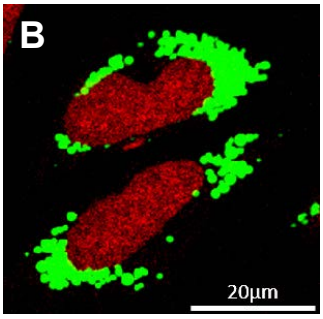
2mg [T3935]

Advantages

- **Maximum excitation wavelength at 404 nm, Maximum emission wavelength at 473 nm (in *n*-hexane)**
- **Strong and specific fluorescence imaging of lipid droplets**
- **Low cytotoxicity**

Application: Intracellular Lipid Droplet Imaging





(Observation via Leica TCS SP5)

[Cell Pre-Treatment]

(A) 3T3-L1 cells were incubated with differentiation medium in a 5% CO_2 incubator at 37°C for 10 days.

(B) HeLa cells were incubated with oleic acid in a 5% CO_2 incubator at 37°C for 48 hours.

[Preparation of ShoyakuGreen Solution]

Stock solution: ShoyakuGreen was dissolved in 100% DMSO at a concentration of 1 mM.

Working solution: The above stock solution was diluted to a final working concentration of 1 μM in culture medium.

[Fluorescence Staining of Intracellular Lipid Droplets]

1. Remove culture medium and Replace with 1 μM ShoyakuGreen working solution (Green).
2. Incubate cells in a 5% CO_2 incubator at 37°C for 30 minutes.
3. Wash – PBS x 3.
4. Fixation - 4% PFA/PB, RT, 10 minutes.
5. Wash – PBS x 3.
6. Permeabilization - 0.1% Triton X-100/PBS, RT, 15 minutes.
7. Wash – PBS x 3.
8. RNase Treatment – 100 μg/mL RNase/PBS, 37°C, 20 minutes.
9. Wash – PBS x 3.
10. PI Staining – add 5 μg/mL PI/PBS (red), 15 minutes.
11. Wash – PBS x 3.
12. Observe lipid droplets using an appropriate fluorescence microscope.

Reference Y. Fuchi, K. Hamada, S. Karasawa, *et al.*, *Sci. Rep.* **2019**, *9*, 17723. https://doi.org/10.1038/s41598-019-53882-z

T3935 is commercialized under the instruction of Prof. Satoru Karasawa, Dr. Yasufumi Fuchi, and Dr. Koichi Hamada.

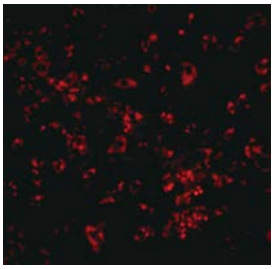
Nile Red [for Biochemical Research]

100mg / 500mg [N1272]

Nile Red is a lipophilic dye widely used to stain lipid droplets. When incorporated into intracellular lipid droplets, it emits strong fluorescence (Ex_{max}: 551 nm, Em_{max}: 631 nm). It can be used on both living and fixed cells.

Application : Lipid Droplet Staining by N1272

1. Culture 3T3-L1 cells and induce differentiation into adipocytes.
2. Remove the medium and wash twice with PBS(-).
3. Fix with 4% PFA for 10 minutes and wash twice with PBS(-).
4. Add PBS(-) containing N1272 dissolved in DMSO to a final concentration of 1 μM and incubate at 37°C for 30 minutes.
5. Remove the staining solution and wash twice with PBS(-).
6. Apply a mounting agent the cells and observe via a fluorescence microscope.



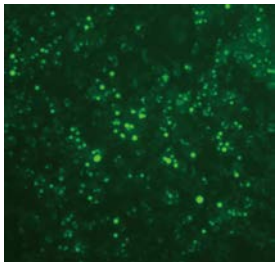
3T3-L1 cells stained by N1272

Pyrromethene 546

1g [D4341]

Application

1. Induce differentiation of 3T3-L1 cells into adipocytes.
2. Add 500 µL of **D4341** solution prepared to 1 µM per well to stain lipid droplets.
3. After staining, fix with 4% PFA and observe.



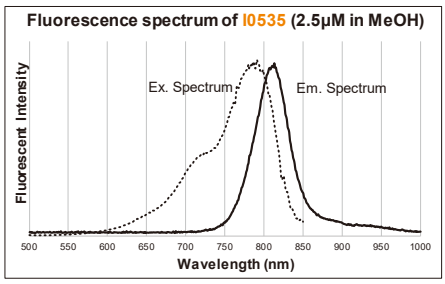
3T3-L1 cells stained by **D4341**

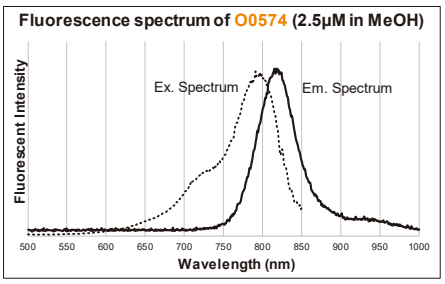
Related Products

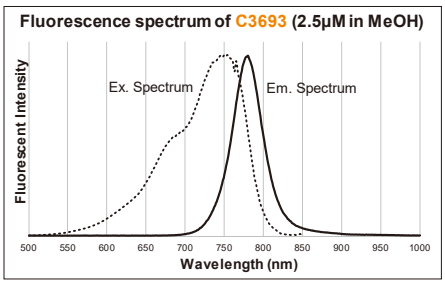
BDP FL 5mg / 25mg [D5554] **BDP FL NHS Ester** 5mg / 25mg [D5555]
BDP FL (1mg×3) 1set [B6272] **BDP FL NHS Ester** (1mg×3) 1set [B6232]

Near-infrared Fluorescent Dyes

Indocyanine Green (= ICG)	100mg / 1g [I0535]
ICG Carboxylic Acid	1g / 5g [O0574]
IR 754 Carboxylic Acid	1g / 5g [C3693]
IR 775 Chloride	1g / 5g [I1026]
IR 783	200mg / 1g [I1031]







Application

Cell imaging using liposomes encapsulating ICG
W. Zheng, M. Wu, *et al.*, *Int. J. Nanomed.* **2022**, *17*, 3217. https://doi.org/10.2147/IJN.S364264

Cell imaging using IR783 incorporated into cancer cells
L. Duan, F. Yan, *et al.*, *Int. J. Clin. Exp. Pathol.* **2019**, *12*, 2353. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6949655/

Cell imaging using antibodies labeled with near-infrared fluorescent dyes
S. Yamashita, M. Kojima, N. Onda, T. Yoshida, M. Shibutani, *Cancer Med.* **2022**, *00*, 1. https://doi.org/10.1002/cam4.5302

Cell imaging using proteins labeled with near-infrared fluorescent dyes
A. Aayush, S. Darji, D. Thompson, *et al.*, *Oncotarget* **2022**, *13*, 1004. https://doi.org/10.18632/oncotarget.28271

Cell imaging using nanoparticles encapsulating near-infrared fluorescent dyes
Y. Tian, P. Huang, G. Lu, *et al.*, *J. Nanobiotechnology* **2021**, *19*, 365. https://doi.org/10.1186/s12951-021-01109-7

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