Near-Infrared Fluorescent Dyes & Luminescent Substrates

Biotium
Glowing Products for Science™

For in vivo imaging and other applications

NIR CF™ Dye Advantages

Bright

NIR CF™ dyes match or surpass Alexa Fluor®, Cy®, and other commercial dyes in brightness

Photostable

CF™ dyes are among the most photostable dyes

Water soluble

Highly water soluble without carrying excessive negative charge, for superior conjugate specificity and *in vivo* half-life

pH Insensitive

Highly fluorescent across a wide pH range

Compatible with popular instruments

Including Caliper IVIS®, LI-COR Odyssey®, Olympus OV-100, and VisEn FMT

NIR CF™ Dye Applications *(see p. 2)*

In Vivo Imaging

CF™ reactive dyes, antibody labeling kits, and conjugates for *in vivo* imaging

Near-IR Westerns

Sensitive and highly linear multiplex Western blotting

In-Cell Western

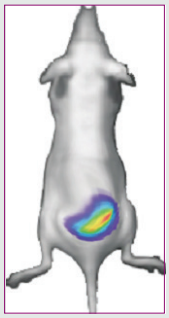
High-throughput, two-color quantitation of protein expression in cells

Flow Cytometry

Superior non-tandem long-wavelength dyes and conjugates

Microscopy

Bright and photostable conjugates and antibody labeling kits for microscopy



Near-Infrared CF™ Dyes

Next generation Near-IR dyes for labeling proteins, nucleic acids, and other biomolecules

Near-infrared (near-IR, NIR) dyes offer important advantages over traditional visible light dyes. Because cellular or tissue components produce minimal autofluorescence in the near-IR region, near-IR dyes have the potential to offer highly specific and sensitive detection in complex biological systems. Also, because light with wavelength in the near-IR region has strong tissue penetration, near-IR dyes are ideal for *in vivo* fluorescence imaging, an emerging field that has advanced rapidly in recent years. Near-IR dyes are also used for highly sensitive multiplex Western blotting and In-Cell Western™ assays.

First generation near-IR dyes suffer from problems of limited fluorescence brightness due to excessive dye aggregation and poor stability. As a result of novel molecular engineering by scientists at Biotium, near-IR CF™ dyes overcome these problems, resulting in several key advantages over other near-IR dyes.

Biotium's near-IR CF™ dyes are exceptionally bright and photostable. For example, our CF™750 is so bright, it can be excited at 633 nm (i.e., at the shoulder wavelength of the absorption maximum) but still emits stronger fluorescence at ~770 nm than APC-based tandem dyes, making the dye particularly useful for flow cytometry applications without the spillover and stability challenges encountered with tandem dyes.

In addition, near-IR CF dyes possess a proprietary structural feature that renders the dyes highly water soluble without introducing excessive negative charge. Consequently, a higher number of near-IR CF dyes can be conjugated to a protein for maximal fluorescence, and the resulting protein conjugate can be expected to have less non-specific binding and a longer *in vivo* half-life. Finally, near-IR CF dyes succinimidyl esters have much higher labeling efficiency than other near-IR dyes because of their excellent solubility and high reactivity (generally >95%).

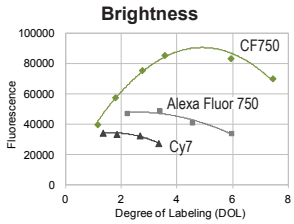


Figure 1. Relative fluorescence of goat anti-mouse IgG conjugates labeled with CF750, Alexa Fluor 750 or Cy7, respectively. Fluorescence values were measured on a Hitachi F-4500 fluorometer.

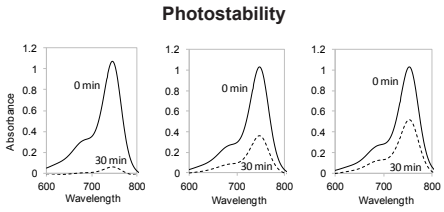


Figure 2. Stability of Cy7, Alexa Fluor 750 and CF750 dyes. Shown are the absorption spectra of the respective dyes before (solid line) and after (dashed line) 30 minutes of exposure to sunlight.

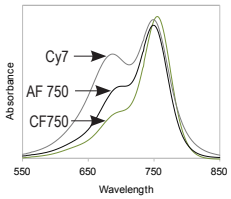


Figure 3. Goat anti-mouse IgG was labeled with CF750, Alexa Fluor 750 or Cy7. Absorbance of the conjugated dyes were normalized to their respective absorbance max. Cy7 and Alexa Fluor 750 have large shoulder peaks, which are indicative of dye aggregation and do not contribute to overall fluorescence intensity.

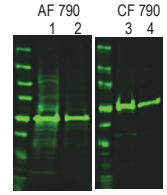


Figure 4. Near-IR CF dyes carry less negative charge than other near-IR dyes, resulting in less non-specific binding of conjugates. Western blotting with near-IR CF dyes, detected using the Odyssey infrared imaging system (LI-COR Biosciences). Two dilutions of HeLa cell lysate (1X and 1:5) were blotted with mouse anti-tubulin antibody followed by goat anti-mouse conjugated to Alexa Fluor 790 (AF790) (1-2) or CF790 (3-4).

Table 1. Near-infrared CF™ Dyes

Dye	Ex/Em (nm)*	Replacement for:	Advantages
CF™680 CF™680R	681/698 680/701	Alexa Fluor® 680, Cy®5.5, DyLight® 680, IRDye® 680LT	• CF™680 is the brightest among spectrally similar 680 nm dyes • CF™680R is the most photostable 680 nm dye • CF™680R is suitable for labeling small molecules like nucleic acids • Compatible with LI-COR Odyssey
CF™750	755/777	Alexa Fluor® 750, Cy®7, DyLight® 750, APC-Alexa Fluor® 750, IRDye® 750	• Exceptionally bright and stable • Highly water soluble without bearing excessive charge • CF™750 has better signal-to-noise ratio compared to APC-Alexa Fluor® 750 with 633 nm excitation • CF™770 is compatible with LI-COR Odyssey®
CF™770	770/797	DyLight® 800, IRDye® 800CW	
CF™790	784/806	Alexa Fluor® 790	

Near-Infrared CF™ Dyes

NEAR-IR CF™ DYE APPLICATIONS

In Vivo Imaging

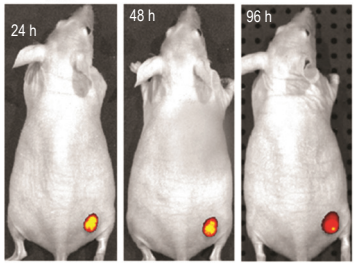


Figure 5. Tumors in mice were imaged using an IVIS imaging system (Caliper Life Sciences) 24 hours, 48 hours, and 96 hours after IV injection of Avastin conjugated to CF750. Images courtesy of Caliper Life Sciences.

Western Blotting

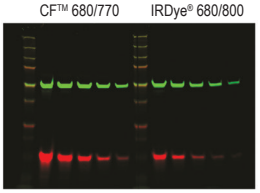


Figure 7. Two-fold dilutions of HeLa cell lysate blotted with mouse anti-tubulin and rabbit anti-COX IV antibodies followed by goat anti-mouse CF770 or IRDye 800 (green) and goat anti-rabbit CF680 or IRDye 680 (red) at the same final concentrations. Membranes were imaged using a LI-COR Odyssey® infrared imaging system. Quantitation of the bands showed approximately a 3.5-fold higher fluorescence intensity of CF dyes compared to the respective IRDyes.

In-Cell Western®

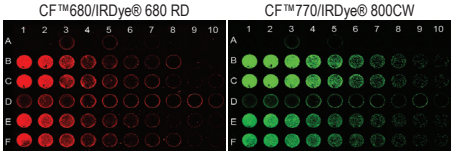


Figure 8. Comparison of CF Dye and IRDye secondary antibody conjugates by In Cell Western™ using the LI-COR Odyssey near-IR imaging system. Two-fold dilutions of HeLa cells grown in 96-well tissue culture plates were fixed, permeabilized and stained with mouse anti-tubulin and rabbit anti-COXIV antibodies, followed by CF680 goat anti-rabbit and CF770 goat anti-mouse (Rows B & C) or IRDye 680RD goat anti-rabbit and IRDye 800CW goat anti-mouse (LI-COR Biosciences) (Rows E & F) at 1 ug/mL. The plate was scanned using a LI-COR Odyssey near-infrared imaging system. Fluorescence quantitation showed that the CF dye conjugate staining had two-fold higher signal compared to the respective IRDye conjugates. Biotium's RedDot™ far-red nuclear stains can be used for cell number normalization for In Cell Western™ assays; visit www.biotium.com for more information.

Fluorescence Microscopy

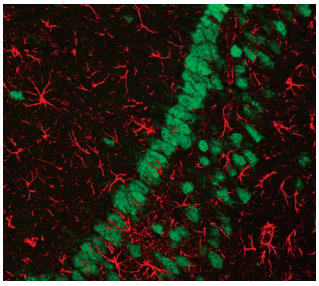


Figure 6. Frozen section of rat brain stained with CF488A-conjugated mouse anti-NeuN antibody (neuronal nuclei, green) and rabbit anti-GFAP followed by CF680 goat anti-rabbit IgG, highly cross-adsorbed (glial cells, red).

Flow Cytometry

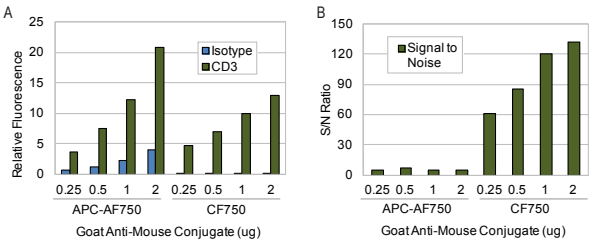


Figure 9. Jurkat cells were stained with isotype or mouse anti-human CD3 antibody followed by goat anti-mouse APC-Alexa Fluor 750 (Invitrogen) or CF750 using the amount of antibody shown. Fluorescence was analyzed using a BD LSR II flow cytometer with 633 nm excitation and 780/60 nm emission filter. A. Relative fluorescence values of the geometric means. B. Signal to noise ratio (CD3 geometric mean/Isotype geometric mean).

Selected References

Abbreviations: ICW: LI-COR In Cell Western®; IHC: immunohistochemistry; WB: Western blotting

Alfonso-Loeches, S, et al. (2012). Glia 60, 948-964. (CF770 succinimidyl ester-labeled antibody; *in vivo* imaging of mouse brain; Caliper IVIS®-200 imaging system)
Alfonso-Loeches, S, et al. (2013). Toxicology. http://dx.doi.org/10.1016/j.tox.2013.03.001 (CF770 succinimidyl ester labeled GFAP and MAP2; *in vivo* imaging of mouse brain; Caliper IVIS®-200 imaging system)
Audebert, M, et al. (2012). Toxicol Appl Pharmacol 260, 58-64. (CF770 goat anti-rabbit IgG; ICW; LI-COR Odyssey®)
Dabek, M, et al. (2011). Inflamm Bowel Dis 17, 1409-1414. (CF770 goat anti-rabbit IgG; WB; LI-COR Odyssey®)
Darniot, M, et al. (2012). Antiviral Res 93, 364-373. (CF750 succinimidyl ester labeling of siRNA; *in vivo* imaging; VisEn Medical FMT 2500 imaging system)
Gauthier, T, et al. (2013). Mol Nutr Food Res DOI 10.1002/mnfr.201200755. (CF680 goat anti-rabbit IgG, CF770 goat anti-rabbit IgG; WB; LI-COR Odyssey®)
Guo, K, et al. (2012). Oncotarget 3: 158-171. (CF750 antibody labeling kit (Caliper); *in vivo* imaging, IVIS® Spectrum Imaging System 3D Series)
Huc, L, et al. (2012). Toxicol In Vitro 26, 709-717. (CF770 goat anti-mouse IgG; WB; LI-COR Odyssey®)
Jamin, EL, et al. (2013). PLoS One 8: e58591. (CF770 goat anti-rabbit IgG; ICW; LI-COR Odyssey®)
Kohler, C, et al. (2012). BMC Microbiol 12, 210-223. (CF680 goat anti-rabbit IgG; WB; LI-COR Odyssey®)
Lucioli, J, et al. (2013). Toxicon http://dx.doi.org/10.1016/j.toxicon.2013.01.024 (CF770 goat anti-mouse IgG, CF770 goat anti-rabbit IgG; WB; LI-COR Odyssey®)
Martinsen, A, et al. (2012). Cell Calcium doi.org/10.1016/j.ceca.2012.07.002 (CF770 goat anti-mouse IgG, CF770 goat anti-rabbit IgG; WB; LI-COR Odyssey®)
Matsuo, H, et al. (2012). Int J Nanomedicine 7, 3341-3350. (CF750 SE; *in vivo* imaging viral particles, Olympus OV-100 imaging system)
Moussa, L, et al. (2012). Clin Nutr. http://dx.doi.org/10.1016/j.clnu.2012.05.021 (CF770 anti-rabbit IgG; WB; LI-COR Odyssey®)
Olivier, I, et al. (2011). Inflamm Bowel Dis 17, 747-757. (CF770 goat anti-rabbit IgG; WB; LI-COR Odyssey®)
Sun, Y. (2012). BioTechniques Rapid Dispatches DOI: 10.2144/000113855. (CF680 succinimidyl ester, CF770 succinimidyl ester; *in vivo* imaging)
Titova, E.and Obenaus, A. (2012). Animal Models of Acute Neurological Injuries II: Injury and Mechanistic Assessments Volume 1, 625-652. (CF680- and CF770- secondary antibody conjugates; IHC; LI-COR Odyssey®)
Tynan, CJ, et al. (2012). PLoS One 7, e36265. (CF790 succinimidyl ester-labeled transferrin; single molecule imaging)
Wu, CL, et al. (2012). PLoS One 7, e34999. doi:10.1371/journal.pone.0034999 (CF680 goat anti-mouse IgG; WB; LI-COR Odyssey®)

Other NIR Dyes and Luminescent Substrates

Near-Infrared Carbocyanine Membrane Dyes

Label cells or liposomes for transplantation, tracking and fusion studies

Carbocyanine dyes label cytoplasmic membranes and intracellular membrane structures efficiently and permanently . They have been used as tracers in cell–cell fusion, cellular adhesion, and cell transplantation and migration applications due to their properties of low cytotoxicity and high resistance to intercellular transfer. Cells stained with carbocyanine dyes can be fixed with formaldehyde and permeabilized with digitonin for subsequent immunostaining.

CellBrite™ NIR680 (Ex/Em: 683/724 nm), CellBrite™ NIR750 (748/780 nm), CellBrite™ NIR770 (767/806 nm) and CellBrite™ NIR790 (786/820 nm) are novel near-infrared carbocyanine dye for labeling cell membranes. Their fluorescence emission can be imaged by confocal microscopy or near-infrared *in vivo* imaging, allowing researchers to assess cellular labeling *in vitro* by microscopy prior to small animal injection. CellBrite™ NIR dyes are non-toxic, photostable and thermostable. The dyes possess long hydrophobic chains to ensure stable cytoplasmic membrane labeling with minimal dye transfer between cells. Furthermore, the dyes are uniquely structured to have sufficient water solubility so that dye aggregation and precipitation is minimized during cell labeling.

DiR is a classic near-infrared fluorescent lipophilic carbocyanine dye (Ex\Em: 748/780 nm) that has been used to label cells and liposomes for *in vivo* imaging.

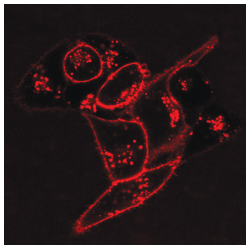


Figure 10. Live HeLa cells stained with CellBrite™ NIR680 dye and imaged with a Zeiss LSM 700 confocal microscope.

Selected References

Jiang, X, et al. (2013). Biomaterials 34, 2969-2979. (DiR-labeled nanoparticles, *in vivo* brain imaging)
Kuai, R, et al. (2011). Mol Pharm. 8(6), 2151-61. (DiR-labeled liposomes, *in vivo* tumor imaging)
Oura, R, et al. (2013). J Immunol 190, 578-585. (DiR-labeled T-cells, *in vivo* imaging)
Ruan, J, et al. (2012). Theranostics 2(6), 618-628. (DiR-labeled embryonic stem cells, *in vivo* imaging gastric cancer)

Luminescent Enzyme Substrates

Image cells expressing luciferase for cell transplantation/migration, xenograft, and reporter gene studies
Non-invasive calcium imaging in cells expressing aequorin

Luminescent enzymes are commonly used reporter genes *in vitro*, and recently have proved to be highly sensitive tools for small animal imaging as well, due to the absence of endogenous luciferase activity in mammals, and because cells and tissue exhibit very low autoluminescence. Biotium offers high purity D-luciferin and coelenterazine luminescent substrates.

D-Luciferin is a substrate for the widely-used reporter enzyme firefly luciferase. The enzyme catalyzes ATP-dependent D-luciferin oxidation to oxyluciferin, producing light emission centered at 560 nm.

Coelenterazine is a substrate for *Renilla* (sea pansy) luciferase, as well as other enzymes such as *Gaussia*, *Metridia*, and *Oplophorus* luciferases. *Renilla* luciferase catalyzes coelenterazine oxidation by oxygen to produce light, and is a widely used reporter gene for luminescence based assays. Coelenterazine native is the natural substrate for *Renilla* luciferase. In addition, over a dozen of coelenterazine analogs have been synthesized that can function as substrates for *Renilla* luciferase, and have different luminescent properties, summarized in Table 2. In addition to quantum yields, emission wavelength can be an important factor when luciferase is used in combination with a fluorescent protein such as GFP for bioluminescent resonance energy transfer (BRET), an important application for the studies of protein-protein interactions.

Coelenterazine and its analogs also bind the jellyfish protein apoaequorin to form aequorin, a calcium-sensing photoprotein. Aequorin can be used for bioluminescent detection of calcium with high sensitivity and dynamics. Compared with fluorescent calcium indicators, aequorin has several advantages in detecting calcium. One major advantage is that the aequorin complex can detect a broad range of calcium concentrations, from ~0.1 µM to >100 µM. Another advantage is that the aequorin complex is stably retained inside cells, making it possible to follow calcium concentration changes for hours to days. Table 3 lists the luminescent properties of coelenterazine analogs in complex with apoaequorin.

Coelenterazines are poorly water soluble, which complicates the formulation of these substrates for use in live animals. Aquaphile™ coelenterazines are specially formulated to readily dissolve in water or buffer for *in vivo* dosing.

Selected References

Jo, M, et al. (2012). Tissue Eng. Regen. Med. 9(3), 157-169. (Coelenterazine; *Gaussia* luciferase reporter gene, *in vivo* cell transplantation, neuronal differentiation)
Naumann, EA, et al. (2010). Nat Neurosci. 13(4): 513–520. (Coelenterazine; aequorin reporter gene, neural activity in freely behaving zebrafish)
Pichler, A, et al. (2005). Clin Cancer Res 11(12), 4487-4494. (D-luciferin, coelenterazine; firefly and *Renilla* fusion proteins, *in vivo* liver transfection)
Ray, P, et al. (2004). Cancer Res. 64, 1323–1330, (Coelenterazine, *Renilla* luciferase, tumor cell metastasis)
Volk-Draper, LD, et al. (2012). Neoplasia 14(10), 926–942. (Coelenterazine; *Renilla* luciferase, tumor xenografts)
Zhao, H, et al. (2004). Mol. Imaging 3 (1), 43 – 54. (D-luciferin, coelenterazine analogs; firefly and *Renilla* luciferases; *in vivo* liver transfection)

Table 2. Luminescent Properties of Coelenterazine Analogs with *Renilla* Luciferase*

Analog	λ_{em} (nm)	Total Light (%)	Initial Intensity (%)
native	475	100	45
400a	400		
cp	470	23	135
e	418, 475	137	900
f	473	28	45
h	475	41	135
n	475	47	900

* Data from Inouye, S and Shimomura, O. (1997). Biochem. Biophys. Res. Commun. 233, 349-353.

Table 3. Luminescent Properties of Coelenterazine Analogs with Apoaequorin*

Analog	λ_{em} (nm)	Relative luminescence capacity	Relative intensity	Half-rise time (s)
native	465	1.0	1.00	0.4-0.8
cp	442	0.95	15	0.15-0.3
e	405, 465	0.50	4	0.15-0.3
f	473	0.80	18	0.4-0.8
fcp	452	0.57	135	0.4-0.8
h	475	0.82	10	0.4-0.8
hcp	444	0.67	190	0.15-0.3
i	476	0.70	0.03	8
ip	441	0.54	47	1
n	467	0.26	0.01	5

* Data from Shimomura, B, et al. (1989). Biochem. J. 261, 913-920.

Product List

Visit www.biotium.com for current pricing

Near-Infrared CF™ Dye Products	Unit Size	Dye	Cat. No.
CF™ Dye Succinimidyl Esters For labeling free amines of target molecules, such as lysine residues in proteins.	1 umol	CF680	92139
		CF680R	92107
		CF750	92142
		CF770	92150
	0.25 umol	CF790	92155
CF™ Dye SE Protein Labeling Kits CF Dye SE kits include all dyes, buffers, and vials required to perform three labeling reactions of 1 mg protein each, with microcentrifuge ultrafiltration vials for quick and easy purification.	3 labelings	CF680	92220
		CF680R	92226
		CF750	92221
		CF770	92222
VivoBrite™ Rapid Antibody Labeling Kits for Small Animal Imaging VivoBrite™ kits include all components of the SE Protein Labeling Kits, plus syringes, filters, and sterile vials for filter sterilization of the antibody conjugate after labeling and purification.	3 labelings	CF680	92160
		CF750	92161
		CF770	92162
		CF790	92163
Annexin V, preservative-free lyophilized solid Fluorescently-labeled Annexin V binds phosphatidylserine on the surface of apoptotic cells. Preservative-free lyophilized solid compatible with *in vivo* use.	25 ug	CF680	29007
		CF750	29006
		CF770	29046
		CF790	29047

Near-Infrared Carbocyanine Dyes	Unit Size	Cat. No.
CellBrite™ NIR680 Cytoplasmic Membrane Dye, 2 mM in DMSO	100 uL	30070
CellBrite™ NIR750 Cytoplasmic Membrane Dye, 2 mM in DMSO	100 uL	30077
CellBrite™ NIR770 Cytoplasmic Membrane Dye, 2 mM in DMSO	100 uL	30078
CellBrite™ NIR790 Cytoplasmic Membrane Dye, 2 mM in DMSO	100 uL	30079
DiR (DiIC18(7)	25 mg	60017

CF dye technology is covered by pending US and international patents; Alexa Fluor is a registered trademark of Molecular Probes, Inc.; CYDYE is a registered trademark of GE Healthcare; DyLight is a trademark of Pierce Biotechnology; LI-COR, Odyssey, In-Cell Western and IRDye are trademarks or registered trademarks of LI-COR, Inc. in the United States and other countries; DeepBlue C is a trademark of BioSignal Packard.

Luminescent Substrates	Unit Size	Cat. No.
D-Luciferin, potassium salt	10 mg	10101
	50 mg	10101-1
	1 g	10101-2
D-Luciferin, sodium salt	10 mg	10102
	50 mg	10102-1
	1 g	10102-2
Coelenterazine native	50 ug	10110
	250 ug	10110-2
	1 mg	10110-1
Coelenterazine *cp*	50 ug	10112
	250 ug	10112-2
	1 mg	10112-1
Coelenterazine *f*	50 ug	10114
	250 ug	10114-2
	1 mg	10114-1
Coelenterazine *fcp*	50 ug	10117
	250 ug	10117-2
	1 mg	10117-1

Luminescent Substrates	Unit Size	Cat. No.
Coelenterazine *h* (2-(4-Dehydroxy) coelenterazine)	50 ug	10111
	250 ug	10111-2
	1 mg	10111-1
Coelenterazine *hcp*	50 ug	10113
	250 ug	10113-2
	1 mg	10113-1
Coelenterazine *i*	50 ug	10121
	250 ug	10121-2
	1 mg	10121-1
Coelenterazine *ip*	50 ug	10116
	250 ug	10116-2
	1 mg	10116-1
Coelenterazine *n*	50 ug	10115
	250 ug	10115-2
	1 mg	10115-1
Coelenterazine 400a (also known as DeepBlue C™)	50 ug	10125
	250 ug	10125-2
	1 mg	10125-1

Luminescent Substrates	Unit Size	Cat. No.
Coelenterazine, 2-methyl analog (Methyl Coelenterazine)	50 ug	10122
	1 mg	10122-1
Coelenterazine Sampler Kit Coelenterazine native, *cp, f, fcp, h, hcp, i, ip*, and *n*	25 ug each	10123
Aquaphile™ Coelenterazine Native	50 ug	10126-50ug
	100 ug	10126-100ug
	5 x 100 ug	10126
Aquaphile™ Coelenterazine *h*	50 ug	10127-50ug
	100 ug	10127-100ug
	5 x 100 ug	10127