Fluorescent Infrared Dyes & Probes

Ultimate Sensitivity & Minimal Background



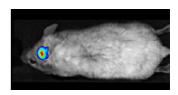
Overview of Infrared Fluorescence Imaging

Fluorescent imaging systems commonly operate in the visible wavelength range between 400 and 700 nm. When working in this scope, biological samples, blotting membranes, plastics and chemical compound libraries for high-throughput screening generate high levels of background interference due to light scattering and autofluorescence. This high background limits the sensitivity of fluorescent systems making it difficult to detect low abundance proteins at endogenous levels. By utilizing fluorescent systems designed to operate within infrared wavelengths between 700 and 1000 nm, both light scattering and autofluorescence are drastically reduced due to the minimal absorption of biological molecules in this region. This results in significantly lower levels of background interference, high signal to background ratios and improved detection sensitivity. AAT Bioquest offers the most complete portfolio of infrared reagents with exceptional brightness and photostability for improved performance in applications such as *in vivo* imaging and Western blotting.

POPULAR APPLICATIONS:

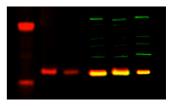
In Vivo Imaging

Maximize the depth of tissue penetration using our comprehensive selection of infrared fluorescent *in vivo* imaging reagents. Develop infrared targeting probes to image a broad range of disease related biomarkers and biological processes to gain a better understanding of disease and therapeutic efficacy.



Infrared Fluorescent Western Blot

Identify specific proteins and precisely measure changes in protein expression against various experimental conditions. Infrared detection is static allowing for a wider linear detection range for improved sensitivity. Signal intensity is unaffected by exposure time and is directly proportional to the amount of target protein in order to produce more consistent and reliable quantitative results.



OTHER APPLICATIONS:

- · Fluorescence microscopy
- · Protein arrays

- In-Cell ELISA
- · Tissue section imaging
- Multiplex Western blots
- Screening of small molecule libraries

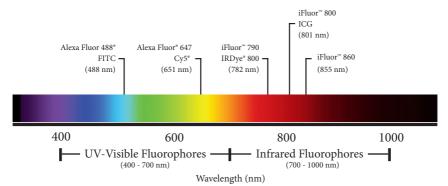


Figure 1. Various fluorophores and their respective excitation wavelengths. In the UV-visible range cellular components, plastics and blotting membranes autofluoresce which can corrupt your image quality and produce interfering effects. In the infrared range autofluorescence from these components are drastically reduced to generate higher signal-to-background ratios and improved detection sensitivity.

In Vivo Imaging: ICG & ICG Derivatives

Indocyanine green (ICG) is a negatively charged, tricarbocyanine dye that is widely exploited for its low toxicity and infrared fluorescence emission. ICG's minimal toxicity is attributed to its strict binding with plasma proteins. This confines it to the vascular compartment where it is rapidly metabolized by the liver and excreted exclusively by the liver and bile ducts. ICG and ICG derivatives (Table 1) can be used as *in vivo* molecular imaging probes. Their infrared fluorescence emission penetrates tissues several millimeters to facilitate deep tissue and small animal *in vivo* imaging. ICG is used in medical diagnostics for determining cardiac output, hepatic function, and liver blood flow and for ophthalmic angiography. In addition, ICG has been conjugated to several antibodies for cancer cell and tumor targeting.

ICG and ICG derivatives

Abs/Em Maxima: 780/800 nm

Excitation Laser: 785 nm

Half-life: 150 to 180 seconds

LD₅₀: 50-80 mg/kg for animals

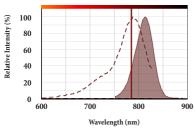


Figure 2. Excitation and emission spectra for ICG. Excitation laser line is set at 785 nm.

Advantages

- Available in a variety of reactive forms for preparing ICG bioconjugates
- Ideal for in vivo imaging
- · Sulfonated ICG for improved aqueous solubility
- Non-sulfonated ICG for applications in organic media
- Compatible with Li-COR* Odyssey* and PerkinElmer* IVIS*

Figure 3. Chemical structure of Indocyanine Green (Cat# 91).

Table 1. Chemical and physical properties of ICG and ICG derivatives.

Cat#	Product Description	Reactivity	Mol. Wt.	Ext. Coeff. 1 (cm-1M-1)	FQY ²	CF at 260 nm ³	CF at 280 nm ⁴
91	Indocyanine Green	Plasma Proteins	774.96	230,000	0.04	N/A	N/A
181	ICG-ATT	NH ₂	760.49	220,000	0.04	0.112	0.072
182	ICG-Osu	NH ₂	828.03	230,000			
180	ICG-Sulfo-Osu	NH ₂	930.07		0.04	0.113	0.073
183	ICG-Sulfo-EG4-OSu	NH ₂	1177.36				
184	ICG-Sulfo-EG8-OSu	NH ₂	1353.57	230,000			
186	ICG Xtra-Osu	NH ₂	1232.62				
189	ICG acid	NH ₂ and OH	844.98	220,000	0.04	0.113	0.073
188	ICG amine	CO₂H	1001.08				
187	ICG maleimide	SH	853.09	230,000			
185	ICG-PEG12-Osu	NH ₂	1427.73				

Note'. 1) Extinction coefficient at their max absorption wavelength; 2) FQY = fluorescence quantum yield in aqueous buffer (pH 7.2); 3) CF at 260 nm is the correction factor used for eliminating the dye contribution to the absorbance at 260 nm (for oligo and nucleic acid labeling); 4) CF at 280 nm is the correction factor used for eliminating dye contribution to the absorbance at 280 nm (for peptide and protein labeling); 5) Hoursescence intensity significantly increases uponly increases uponly increases uponly increases upon upoling to protein and

Antibody & Protein Labeling

Develop custom infrared optical probes for quantitative protein analysis, protein arrays, optical *in vivo* imaging and cell-based assays using AAT Bioquest's infrared iFluor[™] labeling dyes (Table 2). Infrared iFluor[™] dyes maximize assay sensitivity by providing a wide dynamic range for detecting and quantifying low and high abundance proteins. Compared to indirect chemiluminescent detection which is prone to signal saturation and limited by its narrow linear detection range, iFluor[™] infrared fluorescence is static and provides a much wider linear detection range for more accurate analysis.

iFluor™ 750

- Abs/Em Maxima: 749/775 nm
- Excitation Laser: 633, 635, 680 or 685 nm
- Replacement for: Alexa Fluor® 750, Cv®7, DvLight® 750

iFluor™ 790

- Abs/Em Maxima: 782/811 nm
- · Excitation Laser: 785 nm
- Replacement for: Alexa Fluor® 790, IRDye® 800, DyLight™ 800

iFluor™ 800

- Abs/Em Maxima: 801/815 nm
- Excitation Laser: 785 nm
- · Replacement for: Indocyanine Green

iFluor™ 810

- · Abs/Em Maxima: 812/824 nm
- · Excitation Laser: 785 nm

iFluor™ 820

- Abs/Em Maxima: 820/849 nm
- Excitation Laser: 785 nm

iFluor™ 860

- Abs/Em Maxima: 852/877 nm
- Excitation Laser: 785 nm

Advantages

- Exceptionally bright fluorescence and photostability
- High Signal-to-Noise ratio
- Ideal for in vivo molecular imaging, fluorescent Western blots and microscopy
- Compatible with popular instraments such as Li-COR® Odyssey®, PerkinElmer® IVIS® and FMT® systems, Olympus® OV-100™

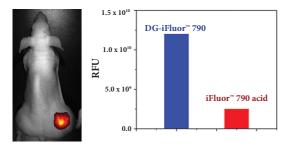


Figure 4. Mice with tumor were intravenously injected with 10 nmol of 2-Deoxyaminoglucose (DG)-Filuor 790 conjugate or control IFluor 790 acid and imaged at the 6th hour. Mouse injected with the DG-IFluor 790 showed specific accumulation of the IFluor 790 probe to the tumor site.

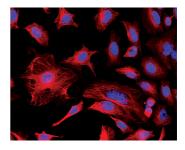


Figure 5. HeLa cells were stained with mouse anti-tubulin followed with iFluor" 790 goat anti-mouse IgG (H+L) (red, Cat# 165007) and counterstained with DAPI (blue, Cat# 17511)

iFluor™ 750 to iFluor™ 860 Dyes

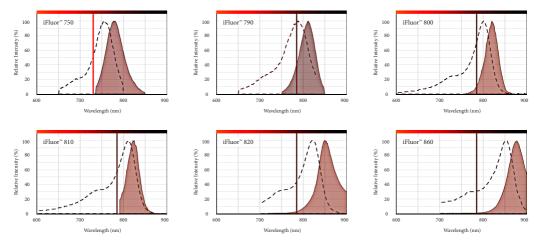


Figure 6. Excitation and emission spectra for infrared iFluor* labeling dyes. Red excitation laser line is set at 730 nm. Black excitation laser line is set at 785 nm.

Table 2. Chemical and physical properties of ICG and ICG analogs.

Cat#	Product Description	Reactivity	Mol. Wt.	Ext. Coeff. 1 (cm-1M-1)	FQY ²	CF at 260 nm ³	CF at 280 nm ⁴
1079	iFluor™ 750 amine	CO₂H	1047.07		0.13	0.025	0.036
1088	iFluor™ 750 hydrazide	Carbonyl	~1100	275,000			
1068	iFluor™ 750 maleimide	SH	~1100	273,000			
1037	iFluor™ 750 succinimidyl ester	Aliphatic amine	~1400				
1360	iFluor™ 790 acid	NH ₂ and OH	1165.29		0.09	0.098	0.091
1362	iFluor™ 790 amine	CO ₂ H	1435.42]			
1364	iFluor™ 790 hydrazide	Carbonyl	1293.34	250,000			
1366	iFluor™ 790 maleimide	SH	1472.51				
1368	iFluor™ 790 succinimidyl ester	Aliphatic amine	1879.45				
1375	iFluor™ 800 acid	NH ₂ and OH	1373.43		0.16	0.184	0.198
1378	iFluor™ 800 maleimide	SH	1617.68	250,000			
1379	iFluor™ 800 succinimidyl ester	Aliphatic amine	1541.91				
1385	iFluor™ 810 acid	NH ₂ and OH	1403.43		0.08	0.206	0.233
1388	iFluor™ 810 maleimide	SH	1525.55	250,000			
1389	iFluor™ 810 succinimidyl ester	Aliphatic amine	1576.03				
1395	iFluor™ 820 acid	NH ₂ and OH	1417.44				
1398	iFluor™ 820 maleimide	SH	1661.69	270,000	N/A	0.132	0.177
1399	iFluor™ 820 succinimidyl ester	Aliphatic amine	1585.92]			
1405	iFluor™ 860 acid	NH ₂ and OH	1403.41		N/A	0.154	0.176
1408	iFluor™ 860 maleimide	SH	1647.66	270,000			
1409	iFluor™ 860 succinimidyl ester	Aliphatic amine	1571.90	1			

Note'. 1) Extinction coefficient at their max absorption wavelength; 2) FQY = fluorescence quantum yield in aqueous buffer (pH 7.2); 3) CF at 260 nm is the correction factor used for eliminating the dye contribution to the absorbance at 260 nm (for oligo and nucleic acid labeling); 4) CF at 280 nm is the correction factor used for eliminating dye contribution to the absorbance at 280 nm (for peptide and protein labeling); 5) Horesexence intensity significantly increases upon upoling to proteins.

Peptide & Oligo Labeling: Tide Fluor™ 7WS & Tide Fluor™ 8 Dyes

Tide Fluor™ 7WS and Tide Fluor™ 8WS dyes (Table 3) are optimized for labeling peptides and oligonucleotides with infrared fluorescence. These infrared probes provide powerful imaging tools to investigate and quantify biological interactions such as second messenger dynamics, enzyme activity, receptor-ligand binding, and protein-protein interactions. Compared to bulky antibodies, small infrared peptide probes facilitate the targeting of receptors in tissues that may be otherwise too difficult to penetrate.

Tide Fluor™ 7WS (TF7WS)

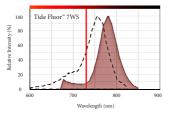
- Abs/Em Maxima: 749/775 nm
- Excitation Laser: 633, 635, 680 or 685 nm
- Replacement for: Alexa Fluor® 750, Cy®7

Tide Fluor™ 8WS (TF8WS)

Abs/Em Maxima: 775/807 nm

Excitation Laser: 785 nm

Replacement for: IRDye® 800



Advantages

- Exceptionally bright fluorescence and photostability
- High Signal-to-Noise ratio
- Optimized for labeling peptides and nucleotides
- Compatible with Li-COR® Odyssey® and PerkinElmer® IVIS®

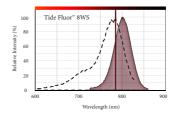


Figure 7. Left) excitation and emission spectra for Tide Fluor "7WS with the excitation laser line set to 730 nm. Right) excitation and emission spectra for Tide Fluor 8WS with the excitation laser line set to 785 nm.

Table 3. Chemical and physical properties of Tide Fluor™ 7WS and Tide Fluor™ 8WS.

Cat #	Product Description	Reactivity	Mol. Wt.	Ext. Coeff. 1 (cm-1M-1)	FQY ²	CF at 260 nm ³	CF at 280 nm ⁴
2304	TF7WS azide	azide click chemistry	959.04		0.12	0.000	0.040
2305	TF7WS alkyne	alkyne click chemistry	928.02				
2330	TF7WS acid	NH ₂ and OH	890.96	275 000			
2331	TF7WS amine	CO₂H	1047.07			0.009	0.049
2332	TF7WS maleimide	SH	1013.08				
2333	TF7WS SE	aliphatic amine	1076.39	9			
2306	TF8WS azide	azide click chemistry	1125.19				
2307	TF8WS alkyne	alkyne click chemistry	1094.18		0.08	0.103	0.190
2335	TF8WS acid	NH ₂ and OH	1057.11	250.000			
2336	TF8WS amine	CO₂H	1213.22	250,000			
2337	TF8WS maleimide	SH	1439.86				
2338	TF8WS SE	aliphatic amine	1343.73				

Note: 1) Extinction coefficient at their max absorption wavelength; 2) FQY = fluorescence quantum yield in aqueous buffer (pH 7.2); 3) CF at 260 mm is the correction factor used for eliminating the dye contribution to the absorbance at 260 nm (for oligo and nucleic acid labeling); 4) CF at 280 nm is the correction factor used for eliminating dye contribution to the absorbance at 280 nm (for peptide and protein labeling); 5) Horosecence intensity significantly increases upon coupling to proteins.

Product Ordering Information For Infrared Dyes and Labeling Kits

Cat #	Product Name	Unit Size	Ex/Em (nm)	Cat #	Product Name	Unit Size	Ex/Em (nm)
189	ICG acid	5 mg	780/800	1366	iFluor™ 790 maleimide	l mg	782/811
188	ICG amine	1 mg	780/800	1368	iFluor™ 790 succinimidyl ester	l mg	782/811
187	ICG Maleimide	l mg	780/800	1375	iFluor™ 810 acid	l mg	809/821
186	ICG Xtra-Osu	l mg	780/800	1378	iFluor™ 810 maleimide	1 mg	809/821
181	ICG-ATT	l mg	780/800	1379	iFluor™ 810 succinimidyl ester	l mg	809/821
182	ICG-OSu	l mg	780/800	1385	iFluor™ 820 acid	1 mg	820/825
185	ICG-PEG12-OSu	l mg	780/800	1388	iFluor™ 820 maleimide	l mg	820/825
183	ICG-Sulfo-EG4-OSu	l mg	780/800	1389	iFluor™ 820 succinimidyl ester	l mg	820/825
184	ICG-Sulfo-EG8-OSu	l mg	780/800	1395	iFluor™ 830 acid	l mg	832/838
180	ICG-Sulfo-OSu	l mg	780/800	1398	iFluor™ 830 maleimide	l mg	832/838
36800	iFluor™ 750 RGD Conjugate	l mg	750/780	1399	iFluor™ 830 succinimidyl ester	l mg	832/838
16506	iFluor™ 750 goat anti-mouse IgG	200 μg	753/779	1405	iFluor™ 860 acid	l mg	863/868
16748	iFluor™ 750 goat anti-mouse IgG	l mg	753/779	1408	iFluor™ 860 maleimide	l mg	863/868
16586	iFluor™ 750 goat anti-mouse IgG	200 μg	753/779	1409	iFluor™ 860 succinimidyl ester	1 mg	863/868
16788	iFluor™ 750 goat anti-mouse IgG	l mg	753/779	91	Indocyanine Green	10 mg	780/800
16660	iFluor™ 750 goat anti-rabbit IgG	200 μg	753/779	23130	Phalloidin-iFluor™ 750 Conjugate	300 Tests	752/778
16813	iFluor™ 750 goat anti-rabbit IgG	1 mg	753/779	23131	Phalloidin-iFluor™ 790 Conjugate	300 Tests	787/808
16720	iFluor™ 750 goat anti-rabbit IgG	200 μg	753/779		ReadiLink™ Rapid iFluor™ 750	2 Labelings	753/779
16842	iFluor™ 750 goat anti-rabbit IgG	1 mg	753/779	1250	Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg		
1088	iFluor™ 750 hydrazide	1 mg	749/775		Antibody Per Reaction*		
1068	iFluor™ 750 maleimide	1 mg	749/775		ReadiLink™ Rapid iFluor™ 790	2 Labelings	782/811
1037	iFluor™ 750 succinimidyl ester	1 mg	749/775	1265	Antibody Labeling Kit *Microscale Optimized for Labeling 50 µg		
16973	iFluor™ 750-streptavidin conjugate	200 μg	753/779		Antibody Per Reaction*		
16999	iFluor™ 750-streptavidin conjugate	1 mg	753/779	2330	Tide Fluor™ 7WS acid	10 mg	749/775
1360	iFluor™ 790 acid	5 mg	782/811	2305	Tide Fluor™ 7WS alkyne	l mg	749/775
1362	iFluor™ 790 amine	1 mg	782/811	2331	Tide Fluor™ 7WS amine	1 mg	749/775
16507	iFluor™ 790 goat anti-mouse IgG	200 μg	782/811	2304	Tide Fluor™ 7WS azide	l mg	749/775
16750	iFluor™ 790 goat anti-mouse IgG	1 mg	782/811	2332	Tide Fluor™ 7WS maleimide	1 mg	749/775
16587	iFluor™ 790 goat anti-mouse IgG	200 μg	782/811	2333	Tide Fluor™ 7WS, succinimidyl ester	1 mg	749/775
16790	iFluor™ 790 goat anti-mouse IgG	1 mg	782/811	2335	Tide Fluor™ 8WS acid	10 mg	775/807
16661	iFluor™ 790 goat anti-rabbit IgG	200 μg	782/811	2307	Tide Fluor™ 8WS alkyne	1 mg	775/807
16815	iFluor™ 790 goat anti-rabbit IgG	l mg	782/811	2336	Tide Fluor™ 8WS amine	1 mg	775/807
16721	iFluor™ 790 goat anti-rabbit IgG	200 μg	782/811	2306	Tide Fluor™ 8WS azide	1 mg	775/807
16843	iFluor™ 790 goat anti-rabbit IgG	l mg	782/811	2337	Tide Fluor™ 8WS maleimide	1 mg	775/807
1364	iFluor™ 790 hydrazide	l mg	782/811	2338	Tide Fluor™ 8WS, succinimidyl ester	l mg	775/807



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