SETA BioMedicals
Fluorescent Tools for BioMedical Applications

http://www.setabiomedicals.com
e-mail: info@setabiomedicals.com

Product number: K8-1342
Product name: Seta-670-NHS

General Data

Molecular Mass:	966.07
Solubility:	Water, Alcohol, DMF, DMSO
Insoluble:	Acetone, Chloroform, Toluene
Storage:	Store out of light, desiccated and refrigerate

Description

- High hydrophilic, amine-reactive fluorescent label containing one reactive NHS-ester group

Applications

- Covalent labeling of proteins, amino-modified DNA and amino-modified oligonucleotides
- Fluorescence Lifetime Label — this label exhibits a distinct lifetime change upon binding to a biomolecule
- Fluorescence Resonance Energy Transfer (FRET) applications
- Single Molecule Applications — **Seta-670** shows extreme low blinking in single molecule measurements
- Flow Cytometry
- Immunofluorescence
- Gene Expression
- Homogeneous Assays
- Assessment of protein structure

Advantages

- Perfectly suited for excitation with the 380, 404, 635, 670-nm diode lasers, LEDs, and UV light
- Sensitive; high extinction coefficients and high quantum yields up to 50% after covalent attachment to proteins
- Quantum yield is highly increased after covalently attachment to proteins and other biomolecules
- pH-insensitive between pH 3 and pH 10
- Good aqueous solubility; this label does not alter the solubility of the protein conjugate
- High photostability; e.g. compared to fluorescein or **Cy5**™
- Low molecular weight — **Seta** dyes do not add substantial mass to the conjugates
- Ideal for non-radioactive labeling of proteins, amino-modified DNA probes and amino-modified oligonucleotides

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Spectral Data

Solvent System: phosphate buffer pH 7.4

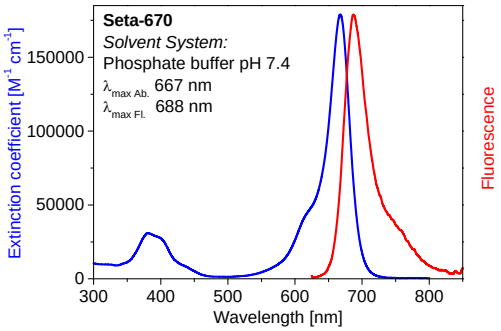
Sample	Dye-to-protein Ratio	Absorption max. [nm]	Extinction Coefficient [$M^{-1}cm^{-1}$]	Fluorescence max. [nm]	Quantum Yield[1] [%]	Luminescence Lifetime at 25 °C [ns]
Free dye	—	667	180,000	688	7	0.42±0.03[2]
BSA conjugate 1	0.5	681		695	45	
BSA conjugate 2	1	681		696	36	
BSA conjugate 3	1.5	681		696	27	2.43±0.03[3]
IgG conjugate 4	1.0	673		693	12	
IgG conjugate 6	5.0	670		693	2	0.85±0.03[4]

[1] Excitation at 635 nm. **Cy5** in phosphate buffer pH 7.4 (QY = 27% [1]) was taking as a reference.

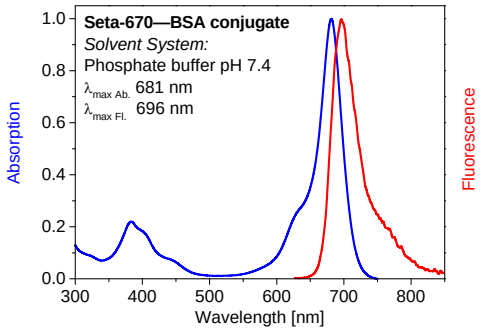
[2] **Seta-670-Carboxy** in phosphate buffer pH 7.4 *vs.* **Alexa 647** in water (1.04 ns [2]); T = 25°C; ISS Chronos FD; excitation 635 nm (laser); bandpass filter 640 nm; longpass filter 670 nm; τ_{mean} **= 0.42 ns**; $\chi^2 = 0.92$; $\tau_1 = 0.38$ ns; $\tau_2 = 1.32$ ns; $f_1 = 0.96$; $f_2 = 0.04$.

[3] **Seta-670—BSA conjugate (D/P = 1.5)** in phosphate buffer pH 7.4 *vs.* **Alexa 647** in water (1.04 ns [2]); T = 25°C; ISS Chronos FD; excitation 635 nm (laser); bandpass filter 640 nm; longpass filter 670 nm; τ_{mean} **= 2.43 ns**; $\chi^2 = 2.17$; $\tau_1 = 0.71$ ns; $\tau_2 = 3.12$ ns; $f_1 = 0.29$; $f_2 = 0.71$.

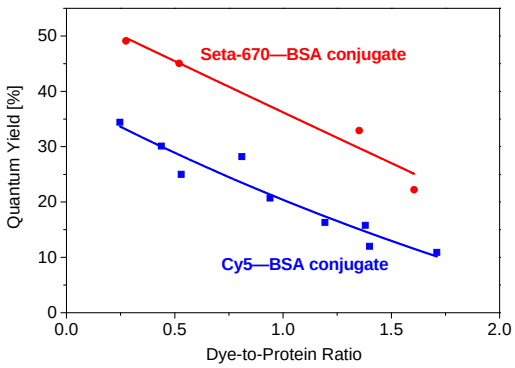
[4] **Seta-670—IgG conjugate (D/P = 5.0)** in phosphate buffer pH 7.4 *vs.* **Alexa 647** in water (1.04 ns [2]); T = 25°C; ISS Chronos FD; excitation 635 nm (laser); bandpass filter 640 nm; longpass filter 670 nm; τ_{mean} **= 0.85 ns**; $\chi^2 = 2.35$; $\tau_1 = 0.26$ ns; $\tau_2 = 2.13$ ns; $f_1 = 0.69$; $f_2 = 0.31$.



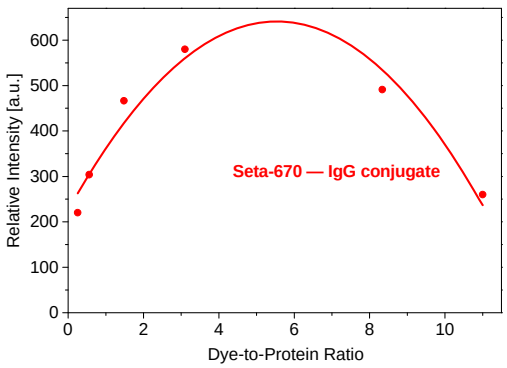
Absorption and emission spectrum of **Seta-670** in phosphate buffer (pH 7.4)



Absorption and emission spectrum of **Seta-670 — BSA conjugate** in phosphate buffer (pH 7.4) (Dye-to-protein ratio 1.0)



Quantum Yield *vs.* Dye-to-protein Ratio of **Seta-670 — BSA conjugates** in phosphate buffer (pH 7.4)



Relative Intensity *vs.* Dye-to-Protein Ratio of **Seta-670 — IgG conjugates** in phosphate buffer (pH 7.4)

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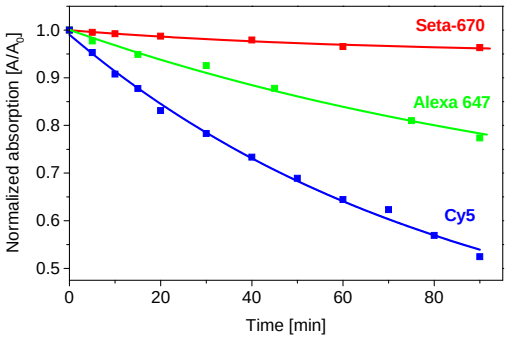
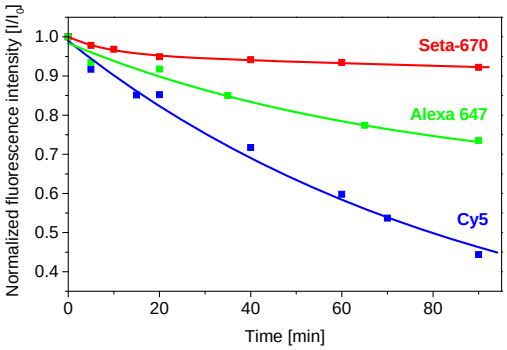
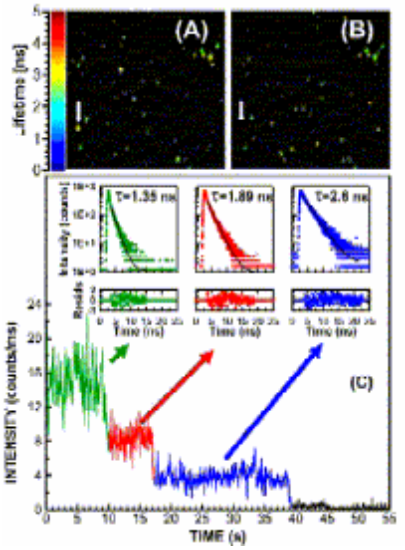


Relative decrease of the long-wavelength absorption band of **Seta-670** as compared to **Cy5** and **Alexa 647** upon irradiation with a Xenon lamp



Relative decrease of the emission of **Seta-670** as compared to **Cy5** and **Alexa 647** upon irradiation with a Xenon lamp



Single molecule applications: **Seta-670-mono-NHS**, a dye that has been recently used in single molecule, homo-FRET measurements showed a remarkably low blinking effect which is an important factor in such measurements [1].

[1] Luchowski R., Matveeva E.G., Gryczynski I., Terpetschnig E.A., Patsenker L., Laczko G., Borejdo J., Gryczynski Z. Single molecule studies of multiple-fluorophore labeled antibodies. Effect of homo-FRET on the number of photons available before photobleaching. *Current Pharmaceutical Biotechnology*, 9, 411-420 (2008).

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[1] R.B.Mujumdar, L.A.Ernst, S.R.Mujumdar, C.J.Lewis, A.S.Waggoner. Cyanine dye labeling reagents: sulfoindocyanine succinimidyl esters. Bioconjugate Chem. (1993) 4, 105–111.

[2] V.Buschmann, K.D.Weston, M.Sauer. Spectroscopic study and evaluation of red-absorbing fluorescent dyes. Bioconjugate Chem. (2003), 14, 195–204.