SETA BioMedicals
Fluorescent Tools for BioMedical Applications

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

Product number: K8-1384
Product name: Seta-700-NHS

General Data

Molecular Mass: 1052.16 (protonated form)

Solubility: water, alcohol, DMF, DMSO

Insoluble: acetone, chloroform, toluene

Storage: Store in absence of light, desiccated and refrigerate

Description

- Water soluble, 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
- Resonance Energy Transfer (RET)
- Flow Cytometry
- Immunofluorescence
- Gene Expression
- Homogeneous Assays
- Assessment of protein structure

Advantages

- Perfectly suited for excitation with the 380, 405, 650, 680, and 700-nm diode lasers, and UV light
- pH-insensitive between pH 4 and pH 9
- Good aqueous solubility; this label does not alter the solubility of the protein conjugate
- High photostability; e.g. compared to fluorescein or **Cy5**™ and **Cy7**™
- Low molecular weight — **Seta** dyes do not add substantial mass to the conjugates
- Ideal for non-radioactive labeling of proteins, amino-modified probes and amino-modified oligonucleotides

Spectral Data

Sample	Dye-to-protein Ratio	Absorption max. [nm]	Extinction Coefficient [$M^{-1}cm^{-1}$]	Fluorescence max. [nm]	Quantum Yield[1] [%]
Free dye in phosphate buffer pH 7.4	—	687	177,000	703	6
Seta-700—IgG in phosphate buffer pH 7.4	1.0	705		715	4
Seta-700—IgG in phosphate buffer pH 7.4	2.0	705		715	3
Seta-700—IgG in phosphate buffer pH 7.4	3.0	705		715	2

[1] **Cy7** in PBS (QY = 13% [1]) was used as a reference. $\lambda_{Ex.}$ = 680 nm.

[1] Texier I, Goutayer M, Da Silva A, Guyon L, Djaker N, Josserand V, Neumann E, Bibette J, Vinet F (2009) Cyanine-loaded lipid nanoparticles for improved in vivo fluorescence imaging. J Biomed. Opt. 14:054005

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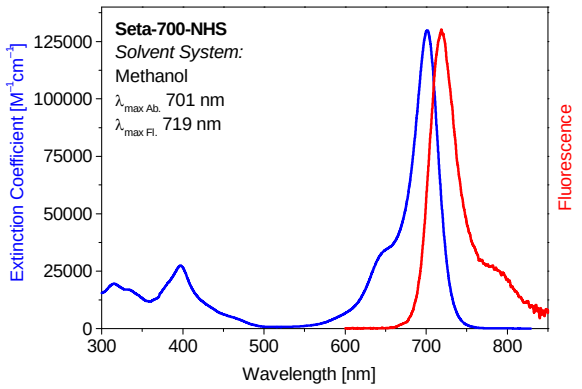
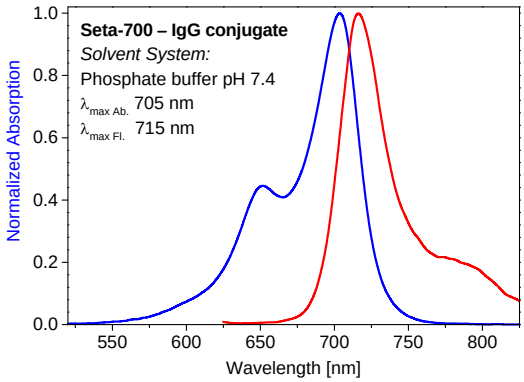
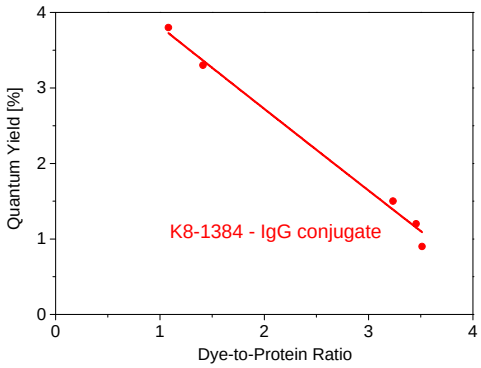


Absorption and emission spectrum of **Seta-700-NHS** in methanol



Absorption and emission spectrum of **Seta-700—IgG** in phosphate buffer (pH 7.4, dye-to-protein ratio 1)



Quantum yield *vs*. dye-to-protein ratio for **Seta-700—IgG conjugates**

Procedure for labeling of protein with Seta-700-NHS ester

A stock solution of 1 mg of the **Seta-700-NHS** in 100 µL of anhydrous dimethyl formamide (DMF) or dimethyl sulfoxide (DMSO) is prepared. Then aliquots of 5, 15, and 30 µL of this dye stock solution are added slowly to a solution of 3 mg of IgG dissolved in 1 mL of a 50 mM bicarbonate buffer (pH 9.0). The mixture is allowed to stir for an additional 2–3 h at 18–25ºC. In most cases the labeling reaction will be completed within 5–10 minutes. The above amounts of NHS ester will yield conjugates with a degree of labeling (DOL) or Dye-to- Protein ratio (D/P) between 1 (5 µL) and 4 (30 µL).

As the number of amino-groups varies with the protein it is important to vary the D/P starting ratios in order to find the appropriate degree of labeling. It is important to note that protein solution used for labeling should be free of amines and TRIS buffer is therefore not suitable as a labeling buffer for NHS-esters. Antibodies stored in buffers containing amines are to be dialyzed against the labeling buffer (phosphate-buffer (PB), or sodium bicarbonate).

Purification of dye-protein conjugates

Separation of the dye–protein conjugate from non-conjugated dye is achieved using gel permeation chromatography on a 1.5 × 25 cm column (stationary phase: Sephadex G-25; eluent: 67 mM PB, pH 7.4). The fraction with the shortest retention time containing the colored dye–protein conjugate is collected. The first colored fraction will be the desired conjugate while the second, slower moving band, in general, contains the unlabeled free dye (hydrolyzed NHS-ester).

Determination of Dye-to-Protein ratio (DP)

The procedure including the x-factor values are provided under Procedures on our website.

Storage of dye-protein conjugates

Dye-protein conjugates should be stored in absence of light, at a temperature of +4 ºC. Sodium azide can be added to avoid bacterial growth.