SETA
BioMedicals
http://www.setabiomedicals.com
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# *Product number:* K8-1252
# *Product name:* Square-635-di-NHS

## General Data

***Molecular Mass:*** 1055.18
995.11 (protonated form)

***Solubility:*** Water, Alcohol, DMF, DMSO

***Insoluble:*** Acetone, Chloroform, Toluene

***Storage:*** Store out of light, desiccated and refrigerate

## Description

Amine-reactive fluorescent label containing two reactive NHS-ester groups.

## 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 635-nm diode laser and 370-nm ultra-bright light emitting diode (LED)
- Sensitive; high extinction coefficients and high quantum yields up to 25% after covalent attachment to proteins
- Low non-specific binding
- 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 — ***Square*** dyes do not add substantial mass to the conjugates
- Ideal for non-radioactive labeling of proteins, amino-modified DNA probes and amino-modified oligonucleotides

## Spectral Data

***Solvent System:*** phosphate buffer, pH 7.4

| Sample | Dye-to-protein Ratio | Absorption max. [nm] | Extinction Coefficient [$M^{-1} \cdot cm^{-1}$] | Fluorescence* max. [nm] | Fluorescence Lifetime [ns] | Quantum Yield [%] |
|---|---|---|---|---|---|---|
| **Free dye** | — | 636 | 216,000 | 648 | 0.36 | 6 |
| **BSA conjugate 1** | 0.5 | 652 | | 665 | | 20 |
| **BSA conjugate 2** | 0.6 | 651 | | 665 | 2.44 | 17 |
| **BSA conjugate 3** | 1.0 | 647 | | 666 | | 4 |

* Excitation at 620 nm

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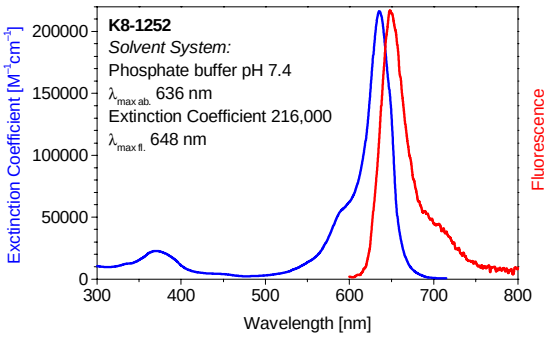
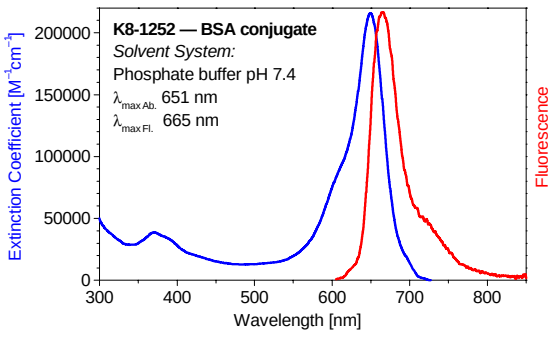
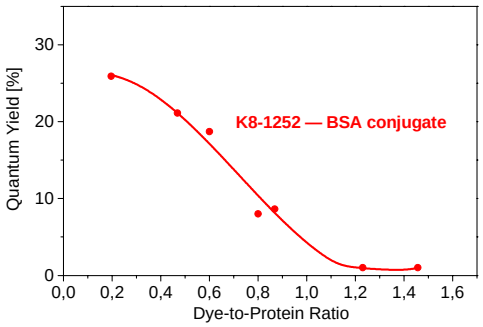



Absorption and fluorescence spectra of **K8-1252** in phosphate buffer (pH 7.4)




Absorption and fluorescence spectra of **K8-1252 — BSA conjugate** in phosphate buffer (pH 7.4, Dye-to-protein ratio 0.6)



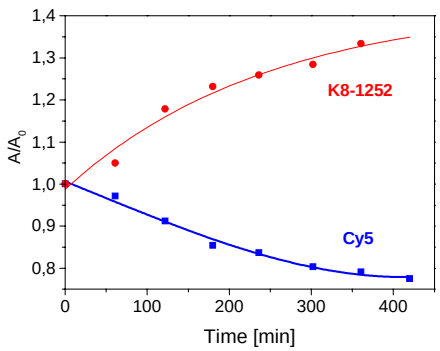

Quantum Yield *vs* Dye-to-protein Ratio of **K8-1252 — BSA conjugates** in phosphate buffer (pH 7.4)

## Photostability

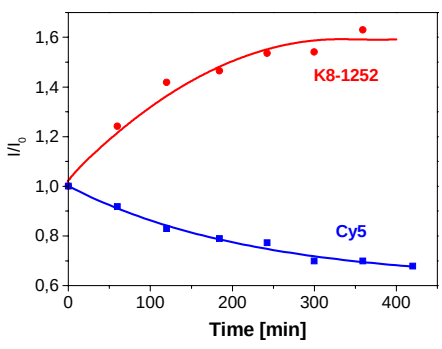
when exposed to light from a lamp (200 W)

***Solvent System:*** phosphate buffer pH 7.4

Due to photochemical transformation **K8-1252** and its conjugates increase brightness during light exposure.




Decay of the long-wavelength absorption band of **K8-1252** as compared to **Cy5**™




Decay of the fluorescence intensity of **K8-1252** as compared to **Cy5**™

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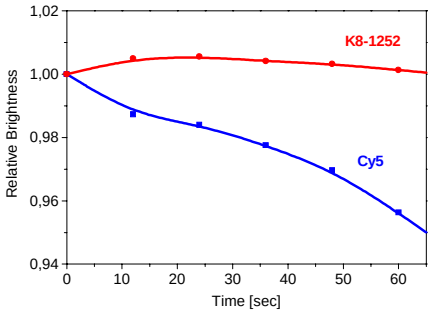


Decay of brightness of a biological image obtained using **K8-1252** as compared to **Cy5**™.
An "Olympus IX-71" fluorescent microscope, a 50% gray filter and a **Cy5**™ filter set were used.