Product number: K8-1407
Product name: Square-650-pH-NHS

General Data

Molecular Mass: 766.84 (protonated form)
Solubility: water, alcohol, DMF, DMSO
Insoluble: acetone, chloroform, toluene
Storage: Store in absence of light, desiccated and refrigerate. Do not store solutions of the free dye as they are not stable. Always use freshly made solutions in DMF or DMSO for labeling. Conjugates are more stable when lyophilized. Minimize light exposure during labeling, purification and storage.

Description

- pH-sensitive, fluorescent label containing one reactive NHS-ester group and pKa in the physiological pH range (pKa = 7.1 - free dye, pKa ~ 6.3 when labeled to antibody).

Applications

- Cell-based imaging applications (ratiometric or FLIM) of e.g. receptor translocations, plasma membrane associated receptor activation or GPCR-ligand interactions via endocytosis.
- Covalent labeling of proteins, amino-modified DNA and amino-modified oligonucleotides and amino-modified lipids.

Advantages

- Perfectly suited for excitation with the 594, 635 and 650-nm diode lasers.
- Sensitive; high extinction coefficients and high quantum yields up to 16% after covalent attachment to biomolecules.
- pH-label that exhibits intensity as well as lifetime-based changes with pH.
- Good aqueous solubility; this label does not alter the solubility of the conjugate.
- Ideal for non-radioactive labeling of proteins, amino-modified DNA probes and amino-modified oligonucleotides.

Spectral Data

<table>
<thead>
<tr>
<th>Sample</th>
<th>Solvent System</th>
<th>Dye-to-Protein Ratio</th>
<th>Absorption max. [nm]</th>
<th>Extinction Coefficient [M⁻¹·cm⁻¹]</th>
<th>Emission max. [nm]</th>
<th>Quantum Yield [%]</th>
<th>Mean Luminescence Lifetime [ns]</th>
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</thead>
<tbody>
<tr>
<td>Free dye</td>
<td>Phosphate buffer pH 5.6</td>
<td>—</td>
<td>653</td>
<td>135,000</td>
<td>671</td>
<td>16</td>
<td>1.17</td>
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<tr>
<td>Free dye</td>
<td>Universal buffer pH 9.0</td>
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<td>535</td>
<td>48,000</td>
<td>663</td>
<td>9</td>
<td>0.53</td>
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<tr>
<td>IgG conjugate 1</td>
<td>Universal buffer pH 2.0</td>
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<td>677</td>
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<td>Phosphate buffer pH 4.0</td>
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<tr>
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</tbody>
</table>

Excitation at 620 nm
**Product number:** K8-1407  
**Product name:** Square-650-pH-NHS

**Absorption and emission spectrum of Square-650-pH in phosphate buffer (pH 5.6)**

**Absorption and emission spectrum of Square-650-pH at pH 5.4 and 9.0**

**Absorption spectra of Square-650-pH as a function of pH**

**Absorption and emission spectrum of Square-650-pH — IgG conjugate in acetate buffered saline (pH 4.0)**

**Relative, pH-dependent emission spectra of Square-650-pH — IgG conjugates (D/P = 0.8)**

**Absorption and emission spectra of Square-650-pH — IgG conjugate (D/P = 0.8) at pH 9 and 2**

**pH-titration curves of Square-650-pH (pKa ~ 7.1): normalized absorption / emission intensity vs. pH**

**Emission spectrum of Square-650-pH vs. pH (λ_exc 589 nm)**
**Product number:** K8-1407  
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**Protein labeling procedure**

A stock solution of 1 mg of the NHS-activated dye in 100–200 mL of anhydrous dimethyl formamide (DMF) or dimethyl sulfoxide (DMSO) was prepared. Then aliquots of 5, 10, 25, and 50 mL of this dye stock solution are added slowly to a solution 5 mg of IgG dissolved in 1 ml of a 50–100 mM bicarbonate buffer (pH 7.5–8.0).

The mixture is allowed to stir for an additional 1–2 h at 25 ºC. However, in most cases the labeling reaction will be completed within 5–10 minutes in particular at higher pH. The lower the pH of the labeling buffer, the longer the reaction will need to complete. Avoid a pH over 8.5 or reduce the labeling time in order to avoid decomposition of the dye. Make sure to protect the labeling solution from excess light. To increase the degree of labeling a higher ratio of NHS-ester vs. protein should be used.

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 (DOL). 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-buffered saline (PBS), or sodium bicarbonate).

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**Storage of dye-protein conjugates**

For short term storage (few hours), the dye-protein conjugates are to be stored under similar conditions as used for the unlabeled protein. Typical storage temperatures are 4 ºC. For long-term storage, prepare smaller aliquots and store the conjugates lyophilized at –20 ºC. Avoid repeated freezing and thawing. Absolutely protect from light.