SETA BioMedicals
Fluorescent Tools for BioMedical Applications

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

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

Sample	Solvent System	Dye-to-protein Ratio	Absorption max. [nm]	Extinction Coefficient [$M^{-1} \cdot cm^{-1}$]	Emission max. [nm]	Quantum Yield[1] [%]	Mean Luminescence Lifetime [ns]
Free dye	Phosphate buffer pH 5.6	—	653	135,000	671	16	1.17
Free dye	Universal buffer pH 9.0	—	535	48,000	663	9	0.53
IgG conjugate 1	Universal buffer pH 2.0	0.8	662		677	7	1.52
IgG conjugate 2	Phosphate buffer pH 4.0	1.0	662		677	10	n/a
IgG conjugate 3	Universal buffer pH 9.0	0.8	544		665 715	9	0.89

[1] Excitation at 620 nm

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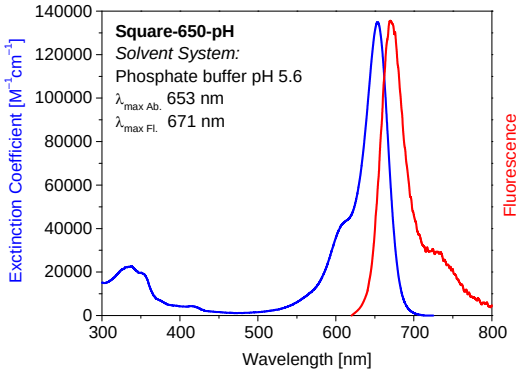
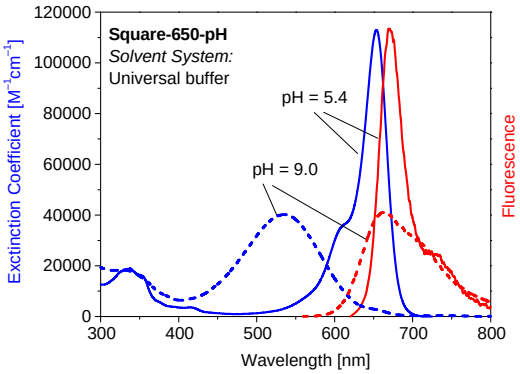
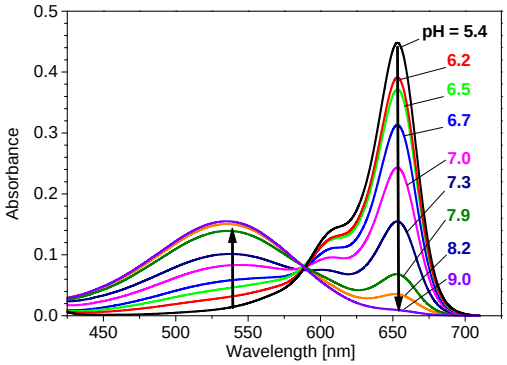


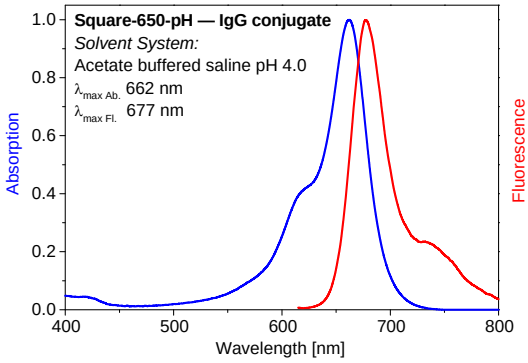
Absorption and emission spectrum of **Square-650-pH** in phosphate buffer (pH 5.6)



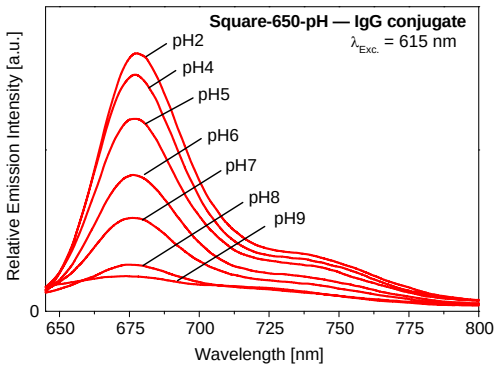
Absorption and emission spectrum of **Square-650-pH** at pH 5.4 and 9.0



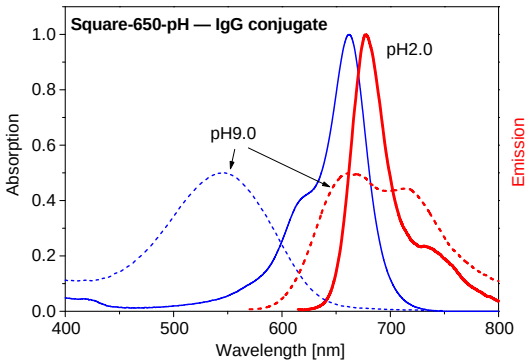
Absorption spectra of **Square-650-pH** as a function of pH



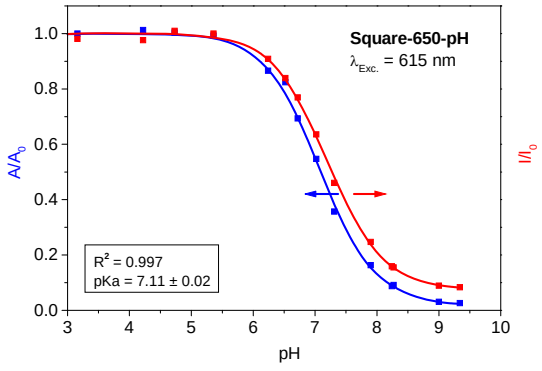
Absorption and emission spectrum of **Square-650-pH — IgG conjugate** in acetate buffered saline (pH 4.0)



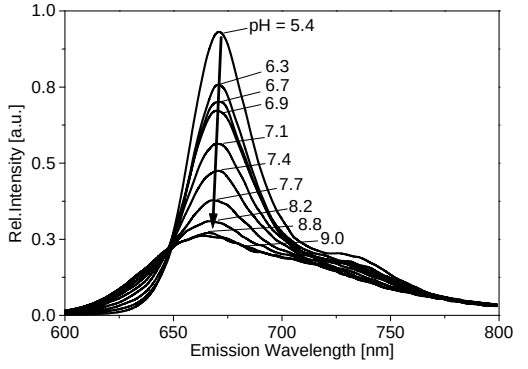
Relative, pH-dependent emission spectra of **Square-650-pH — IgG** conjugates (D/P = 0.8)



Absorption and emission spectra of **Square-650-pH — IgG** conjugate (DP = 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)

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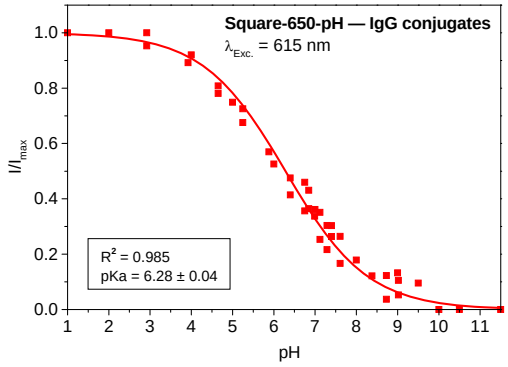
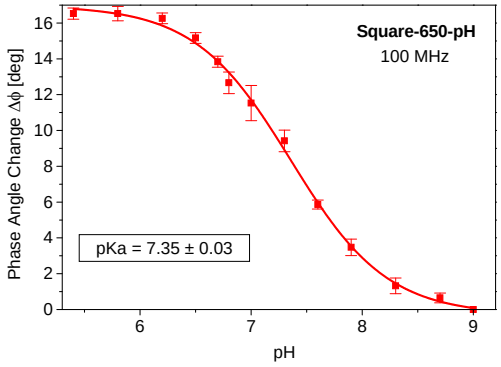
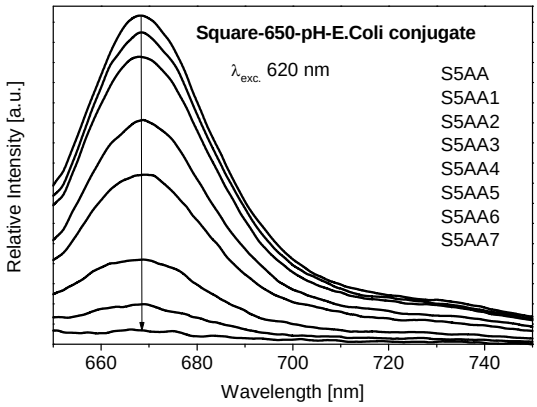


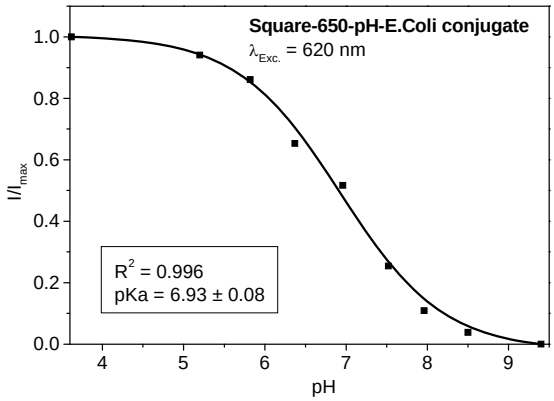
Normalized fluorescence intensity of **Square-650-pH** — IgG conjugate (D/P = 0.8) *vs.* pH (pKa = 6.3)



Changes in phase angel of **Square-650-pH** *vs.* pH, when measured at 100 MHz (pKa = 7.4). Model: Boltzman. χ^2 = 0.11



Emission spectrum of **Square-650-pH** — E.Coli conjugate *vs.* pH



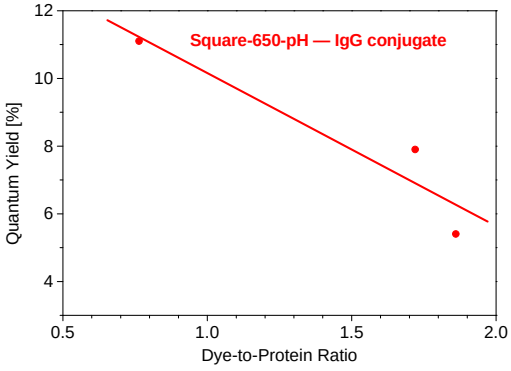
Emission intensity of **Square-650-pH** — E.Coli conjugate *vs.* pH

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).



Quantum yield *vs.* dye-to-protein ratio for **Square-650-pH — IgG conjugates**

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.