

**Product number: K8-1407**

**Product name: Square-650-pH-mono-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.

## Description

pH-sensitive, fluorescent label containing one reactive NHS-ester group and pKa in the physiological pH range (pKa = 7.2 - free dye, pKa ~ 6.1 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

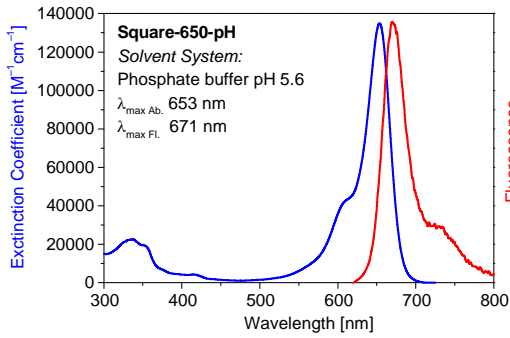
- Perfectly suited for excitation with the 635-nm diode laser
- Sensitive; high extinction coefficients and high quantum yields up to 16% after covalent attachment to biomolecules
- pH-probe that exhibits intensity as well as lifetime-based changes with pH
- Good aqueous solubility; this label does not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

## Spectral Data

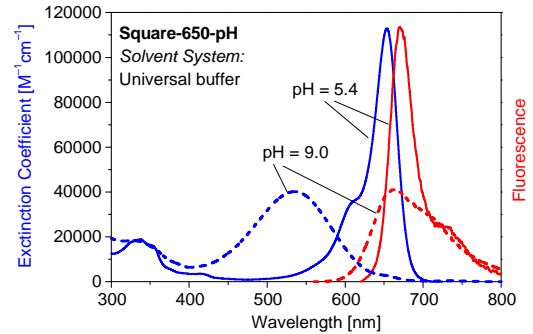
Sample	Solvent System	Dye-to-protein Ratio	Absorption max. [nm]	Extinction Coefficient [ $M^{-1}\cdot cm^{-1}$ ]	Fluorescence max. [nm]	Quantum Yield [%]	Mean Luminescence Lifetime [ns]
Free dye	PB pH 5.6	—	653	135,000	671	16	1.17
Free dye	Universal Buffer pH 9.0	—	535	48,000	663	9	0.53
BSA conjugate 1	PB pH 5.6	0.5				9	n/a
BSA conjugate 2	PB pH 5.6	1.0	670		685	5	n/a
IgG conjugate 1	PB pH 4.0	1.0	662		677	10	n/a
IgG conjugate 2	Universal Buffer pH 2.0	0.8	662		677	7	1.52
IgG conjugate 3	Universal Buffer pH 9.0	0.8	544		665 715	9	0.89

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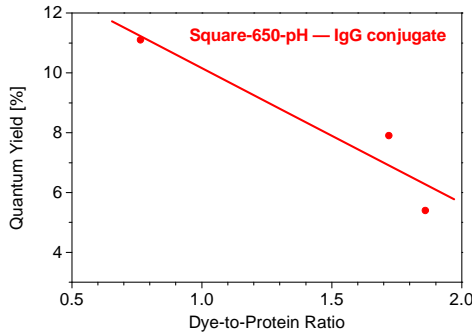
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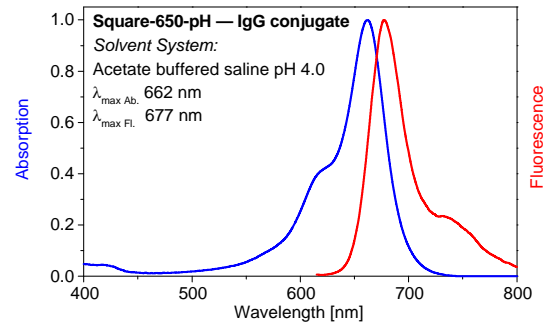
Absorption and emission spectrum of **Square-650-pH** in phosphate buffer (pH 5.6)



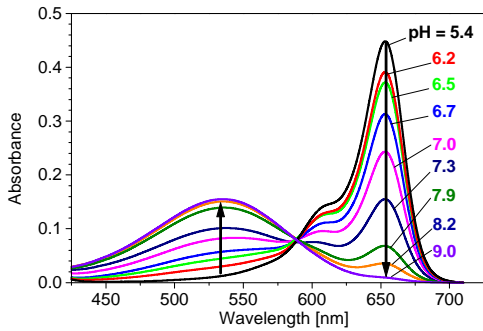
Absorption and emission spectrum of **Square-650-pH** at pH 5.4 and 9.0



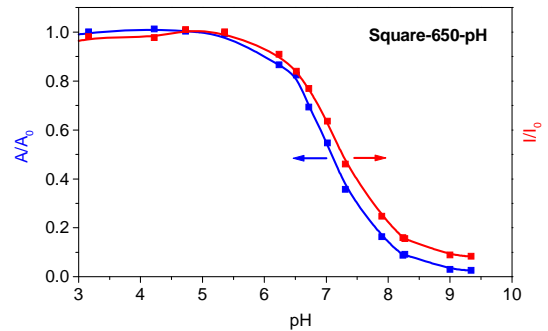
Quantum Yield vs. Dye-to-protein Ratio of **Square-650-pH — IgG conjugates**



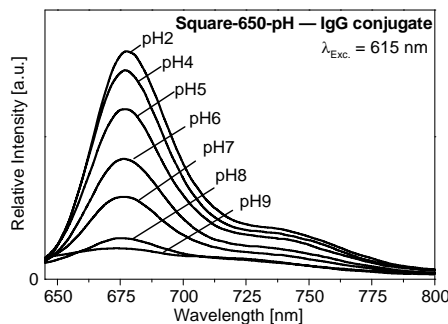
Absorption and emission spectrum of **Square-650-pH — IgG conjugate** in acetate buffered saline (pH 4.0)



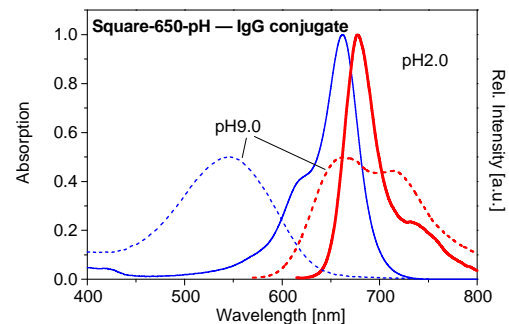
Absorption spectra of **Square-650-pH** as a function of pH



pH-titration curve (normalized fluorescence intensity / absorption vs. pH) of **Square-650-pH** (pKa = 7.2)



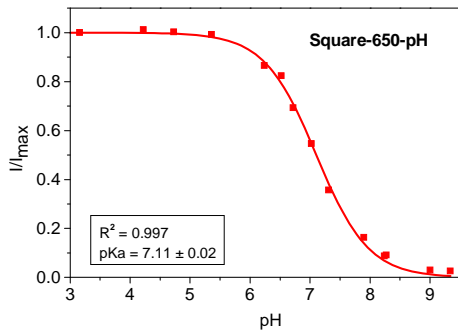
Relative, pH-dependent emission spectra of **Square-650-pH-IgG conjugates** (D/P = 0.8)



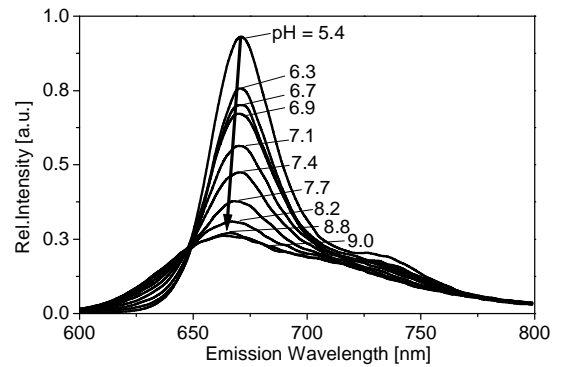
Absorption and emission spectra of **Square-650-pH — IgG conjugate** (DP = 0.8) at pH 9 and 2

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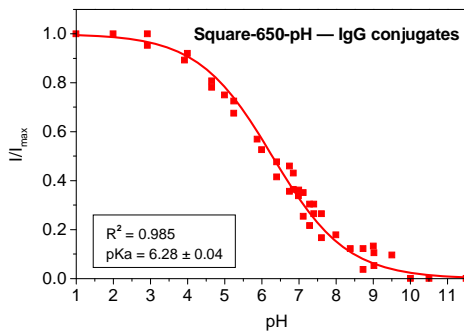
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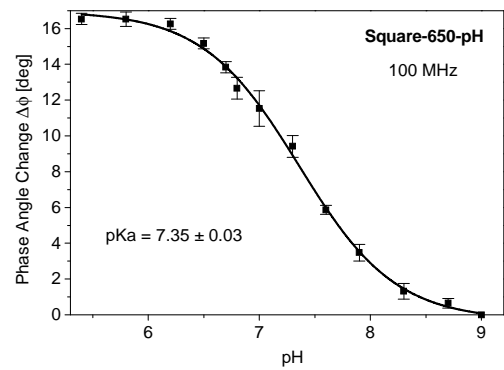
Normalized fluorescence intensity of **Square-650-pH** vs. pH ( $pKa = 7.11$ )



Emission spectrum of **Square-650-pH** vs. pH ( $\lambda_{exc} 589$  nm)



Normalized fluorescence intensity of **Square-650-pH — IgG** conjugate ( $D/P = 0.8$ ) vs. pH ( $pKa = 6.28$ )



Changes in phase angle of **Square-650-pH** vs. pH, when measured at 100 MHz ( $pKa = 7.35$ )