SETA BioMedicals
Fluorescent Tools for BioMedical Applications
http://www.setabiomedicals.com
e-mail: info@setabiomedicals.com

Product number: K8-1352
Product name: Square-660-NHS

General Data

Molecular Mass: 735.81 (protonated form)

Solubility: alcohol, DMF, DMSO, low solubility in water,

Insoluble: acetone, chloroform,toluene

Storage: Store in absence of light, desiccate and refrigerate

Description

- Amine-reactive, lifetime-sensitive fluorescent label containing one reactive NHS-ester group

Applications

- Covalent labeling of proteins, amino-modified DNA and amino-modified oligonucleotides
- Flow Cytometry
- Immunofluorescence
- Gene Expression
- Homogeneous lifetime-based assays
- Assessment of protein structure
- FRET based applications

Advantages

- Perfectly suited for excitation with the 380, 405, 635, 650, and 670-nm diode lasers and UV light
- Sensitive; high extinction coefficients and high quantum yields after covalent attachment to proteins and amino-modified oligonucleotides.
- Low non-specific binding
- Lifetime sensitive label: fluorescence lifetime will change up to 10-times upon binding to a protein
- pH-insensitive between pH 3 and pH 10
- 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 [$M^{-1}cm^{-1}$]	Fluorescence max. [nm]	Quantum Yield[1] [%]	Fluorescence Lifetime at 25 °C [ns]	Polarization at 25 °C [mP]
Free dye	—	657	182,000	676	3	0.27±0.02[2]	323±4[2,3]
BSA conjugate	1.2	676		695	13	3.32±0.03[4]	

[1] Excitation at 620 nm. **Cy5** in phosphate buffer pH 7.4 (QY = 27% [1]) was taking as a reference.

[2] **Square-660-Carboxy** in phosphate buffer pH 7.4 (OD = 0.13) *vs.* **Alexa 647** in water (1.04 ns [2]); T = 25°C; ISS Chronos FD; excitation 635 nm (laser); bandpass filter 640 nm; longpass filter 670 nm; τ_{mean} **= 0.27 ns**; $\chi^2 = 1.78$; $\tau_1 = 0.12$ ns; $\tau_2 = 0.38$ ns; $f_1 = 0.37$; $f_2 = 0.62$.

[3] Excitation between 550–670 nm

[4] **Square-660-Carboxy**—BSA conjugate *vs.* **Alexa 647** in water (1.04 ns [2]); ISS Chronos FD; excitation 635 nm (laser); bandpass filter 640 nm; longpass filter 670 nm; τ_{mean} **= 3.32 ns**; $\chi^2 = 1.70$; $\tau_1 = 0.17$ ns; $\tau_2 = 3.96$ ns; $f_1 = 0.17$; $f_2 = 0.83$.

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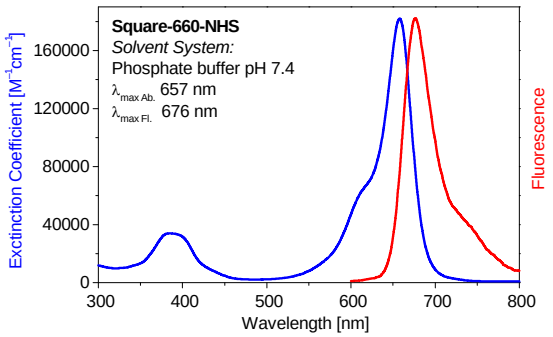
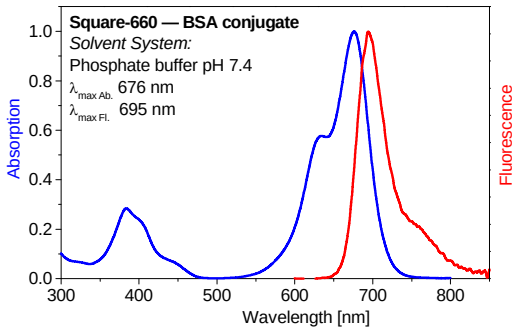
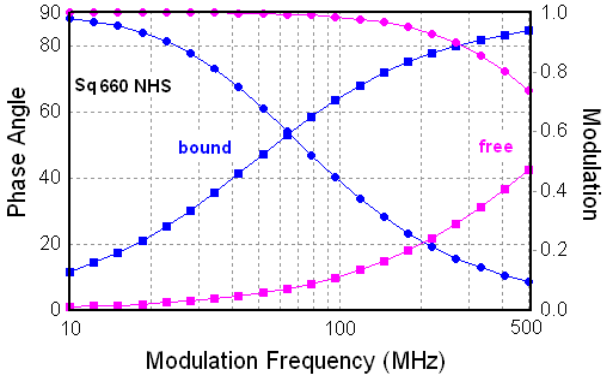


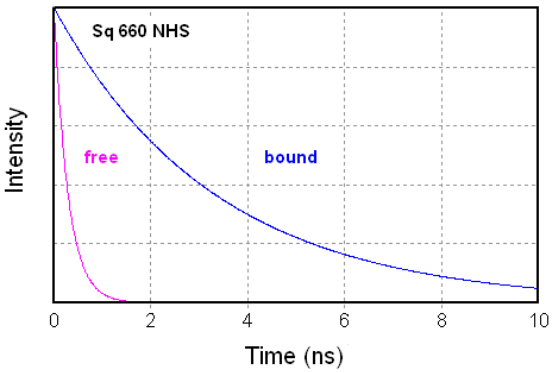
Absorption and emission spectrum of **Square-660-NHS** in phosphate buffer (pH 7.4)



Absorption and emission spectrum of **Square-660 — BSA conjugate** in phosphate buffer (pH 7.4)

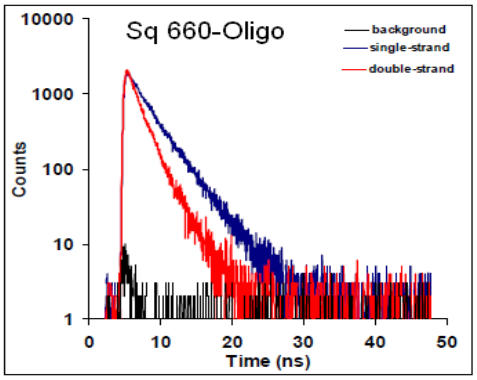


Comparison of the frequency responses of **Square-660** before and after binding to IgG (τ_{free}= 290 ps; τ_{bound}= 2.73 ns)

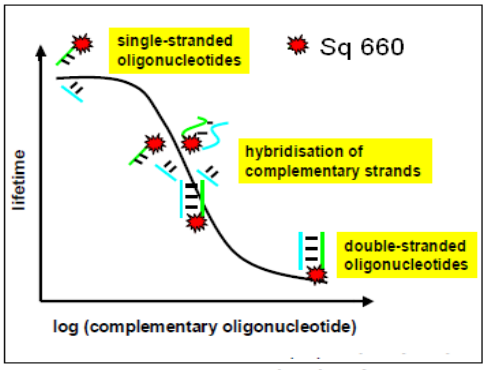


Comparison of the intensity decays of **Square-660** before and after binding to IgG

Lifetime-based Hybridization Assay with **Square-660** labeled oligonucleotide:



Intensity decays of **Square-660 — Oligo** before and after binding to complementary oligonucleotide (τ_{single} = 3 ns; τ_{double} = 1.8 ns)



Lifetime-based detection of SNPs. Detection is based on the change of the fluorescence lifetime of the label **Square-660**.

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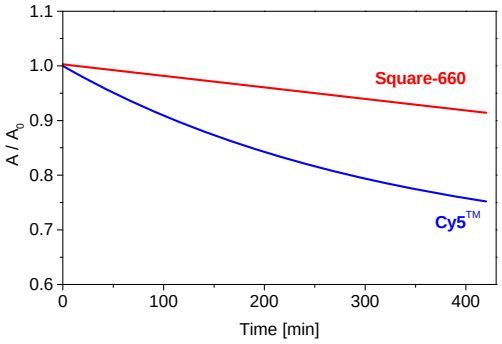
Photostability

when exposed to light from a halogen lamp (200 W)

Solvent System: Phosphate buffer pH 7.4



Decrease of long-wavelength absorption maximum of **Square-660** as compared to **Cy5**™

[1] R.B.Mujumdar, L.A.Ernst, S.R.Mujumdar, C.J.Lewis, A.S.Waggoner. Cyanine dye labeling reagents: sulfoindocyanine succinimidyl esters. Bioconjugate Chem. (1993) 4, 105–111.

[2] V.Buschmann, K.D.Weston, M.Sauer. Spectroscopic study and evaluation of red-absorbing fluorescent dyes. Bioconjugate Chem. (2003), 14, 195–204.