



# SeTau Dyes – Next Generation Long-Wavelength Biomedical Labels with Advanced Characteristics

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## Introduction

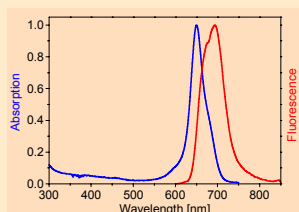
Commercially available dyes of the **Cy** and **Alexa** series have certain shortcomings such as insufficient photo- and chemo-stability, which substantially limits their use in biomedical applications. We introduce the first examples of a new series of next generation dyes — **SeTau** dyes available from SETA BioMedicals — **SeTau-647**, **SeTau-655** and **SeTau-665**, which are water-soluble labels that are extremely bright and promise to overcome the shortcomings of already existing fluorescent labels. The spectral characteristics of these new dyes are summarized in **Table 1** below.

**Table 1.** Spectral characteristics of **SeTau** dyes and the IgG conjugates at dye-to-protein ratios of 1 compared to **Alexa** dyes in phosphate buffer pH 7.4

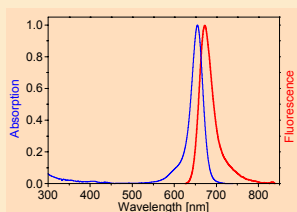
Dye / Conjugate	$\lambda_{\max}$ Ab [nm]	$\lambda_{\max}$ Em [nm]	$\Phi_f$ [%]	$\tau_{\text{mean}}$ [ns]
<b>SeTau-647</b>	650	694	61	3.2
<b>SeTau-647 – IgG conjugate</b>	649	691	47	3.2
<b>SeTau-655</b>	655	673	25	0.9
<b>SeTau-655 – IgG conjugate</b>	657	675	31	1.8
<b>SeTau-665</b>	665	716	62	3.1
<b>SeTau-665 – IgG conjugate</b>	664	709	44	3.2
<b>Alexa 647</b>	649	668	32	1.0
<b>Alexa 647 – IgG conjugate</b>	652	670	42	1.1
<b>Alexa 660</b>	663	690	37	1.2
<b>Alexa 660 – IgG conjugate</b>	663	691	35	-

## Excitation and Emission Spectra

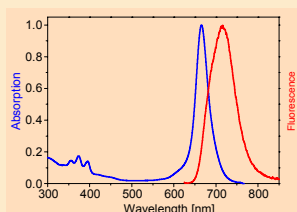
**SeTau** dyes absorb and emit in the red and near-IR spectral range in aqueous media. **SeTau-665** and **SeTau-647** are perfectly excited with common laser sources. Importantly these compounds also exhibit large Stokes' shifts around 40 nm. In the protein (IgG) conjugate the absorption and emission maxima remain almost unchanged and the corresponding shifts do not exceed more than 2 nanometers.



Absorption and emission spectrum of **SeTau-647** in water



Absorption and emission spectrum of **SeTau-655** in water



Absorption and emission spectrum of **SeTau-665** in water

## Large Stokes' Shifts ( $\Delta\nu_{st}$ )

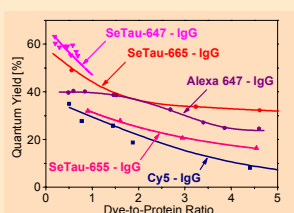
**SeTau-647** and **SeTau-665** exhibit an extremely large Stokes' shift of 44 nm and 51 nm, respectively, which is about 2–3-times larger than those of **Alexa 647** (19 nm) and **Alexa 660** (27 nm).

## High Extinction Coefficients ( $\epsilon$ )

The extinction coefficients of **SeTau** dyes (180,000 – 370,000 M<sup>-1</sup>cm<sup>-1</sup>) are in the same order as for **Cy** and **Alexa** dyes.

## High Quantum Yields ( $\Phi_f$ )

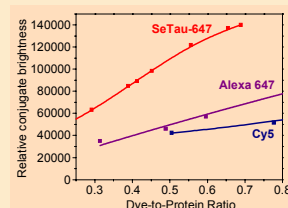
**SeTau-647** and **SeTau-665** are dyes with extremely high quantum yields (see **Table 1**). In general the quantum yields of **SeTau**, **Cy** and **Alexa** dyes do not change upon conjugation to small biomolecules. Importantly for **SeTau-655** and **SeTau-665** the decrease in Q.Y.s after binding to high molecular-weight species such as IgGs is less pronounced as compared to **Alexa** or **Cy** dyes in particular at high D/P ratios (e.g. the Q.Y. of **SeTau-665** is still 32% at a D/P ratio close to 5). **SeTau-647** is an extremely bright label for small molecules (drugs, oligos and phospholipid probes). Its Q.Y. exceeds 60% in water and its extinction coefficient is as high as 368,000 [M<sup>-1</sup>cm<sup>-1</sup>].



Quantum yield vs. dye-to-protein ratio of **SeTau-IgG** conjugates compared to **Cy5** and **Alexa-IgG** conjugates in PB (pH 7.4)

## Extremely Bright

Bio-conjugates of **SeTau** dyes are in general much brighter as compared to those produced with **Cy** and **Alexa** dyes. The **Figure** on right shows that the relative conjugate brightness (quantum yield multiplied by the dye-to-protein ratio (D/P) and extinction coefficient) for **SeTau-647**, **Cy5** and **Alexa 647**.



Relative conjugate brightness of **SeTau-647 – IgG** conjugates in phosphate buffer pH 7.4 as compared to **Cy5** and **Alexa 647** conjugates

**SeTau-647** is an extremely bright label for small molecular weight analytes, while **SeTau-665** shows a **record intensity increase of up to 400 times** in single molecule measurement on silver island films.

## Long Fluorescence Lifetimes

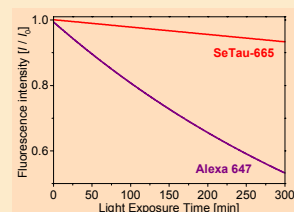
The fluorescence lifetime ( $\tau$ ) of **SeTau-665** in aqueous solutions is about 3 times longer compared to **Cy5** or **Alexa 647**. While the fluorescence lifetimes of **SeTau-665**, **Cy5** and **Alexa 647** do not change noticeably after binding to proteins, the fluorescence lifetime of **SeTau-655 – IgG** conjugate (D/P = 1) is twice higher as compared to the free dye.

## Low Blinking Effect

**SeTau** dyes show low blinking effects and therefore are promising in single molecule applications.

## High Photostability

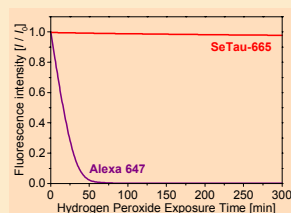
As shown in the **Figure** below, **SeTau** dyes exhibit highly improved photostability compared to **Cy** and **Alexa** dyes. **SeTau-665** shows the most pronounced photostability.



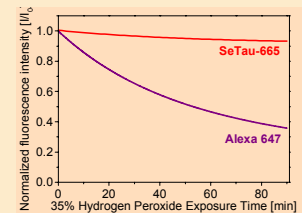
Extreme photostability of **SeTau-665** in comparison to **Alexa 647** in water

## High Stability towards Oxidizing Species

The **Figures** below reveals the extreme chemical stability of **SeTau-665** in bicarbonate buffer at pH 9.4 with 3.5% of hydrogen peroxide in comparison to **Alexa 647**, and the relative stability of **SeTau-665** and **Cy5** in 35% hydrogen peroxide at room temperature.



Extreme stability of **SeTau-665** to 3.5% H<sub>2</sub>O<sub>2</sub> in bicarbonate buffer pH 9.4 in comparison to **Alexa 647**



Decrease in fluorescence intensity of **SeTau-665** as compared to **Alexa 647** in 35% H<sub>2</sub>O<sub>2</sub>

## Conclusion

Dyes of the **SeTau** series are truly advanced labels for biological species such as proteins, immunoglobulins, oligonucleotides, peptides, cells, and drugs and are expected to have widespread use in proteomics, immunology, cytology, genomics, drug screening, cellular and molecular biology, microarrays, and biological imaging. We are currently extending this product-line to other wavelength ranges and functionalities. Please contact us for more information.