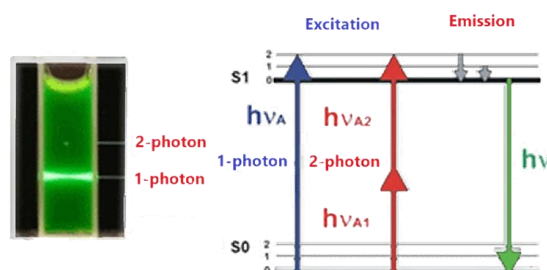




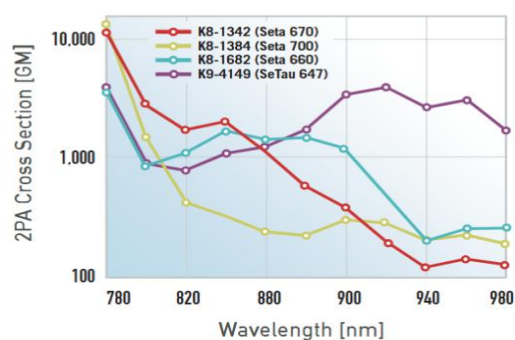
Superior Probes and Labels for Two-Photon Microscopy

Dyes with extremely high Two-Photon-Absorption (2PA) cross sections

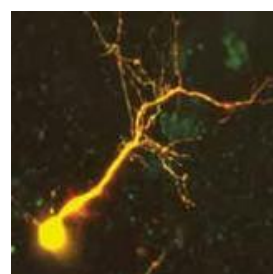
SETA BioMedicals has developed several **Two-Photon-(2P)-probes and labels** based on **squaraines** and **squaraine rotaxanes** of the **Seta** and **SeTau** dye series with extremely high **Two-Photon-Absorption Cross Sections (2PACS)** and emission between 500 nm and 700 nm for in vivo imaging applications.



Nonlinear 2-photon excitation is based on the simultaneous absorption of two photons. Since the energy of a photon is inversely proportional to its wavelength, the two absorbed photons must have a wavelength about twice that of one-photon excitation.



2-photon action cross sections (2PACS)
for several **squaraines** and **squaraine rotaxanes** [1]



2-photon image of neuron loaded with **SeTau-647**

While the **2PACS** cross sections of common fluorescent labels are in the order of 100 GM (**2PACS** of the di-anionic form of fluorescein in water at pH 13 is 36 GM and for Rhodamine B 150 GM for excitation at 800 nm) and even some of the best **2P** labels published have **2PACS** of only several hundred GM, **Seta** and **SeTau** dyes have **2PACS** in the order of **thousand to several thousand GM**.

The **2P wavelengths** and **cross-sections (2PACS)** for selected **Seta** and **SeTau** dyes are provided in the table below.

Product Number (Click for Specs Sheet)	Product Name (Click for Product Info)	Target Group ⁵	Medium	$\lambda_{\max}$ Ab [nm]	Maximum 2PA Wavelength [nm]	$\lambda_{\max}$ Fl [nm]	2P-Excitation Cross-Section [GM]
K7-545	SeTau-425-NHS	NH ₂	PB 7.4	425	n.d.*	545	>100
K7-547	SeTau-405-NHS	NH ₂	PB 7.4	405	750-850	518	>100
K7-567	SeTau-405-Azide	triple-CC	PB 7.4	405	n.d.*	518	>100
K8-1341	Seta-670-Maleimide	SH	PB 7.4	667	n.d.*	688	~1,925
K8-1342	Seta-670-NHS	NH ₂	PB 7.4	667	840	688	~1,925
K8-1346	Seta-670-Azide	triple-CC	PB 7.4	667	n.d.*	686	~1,925
K8-1351	Square-660-Carboxy	Probe [†]	PB 7.4	657	n.d.*	676	~1,925
K8-1352	Square-660-NHS	NH ₂	PB 7.4	657	n.d.*	676	~1,925
K8-1355	Square-680-Carboxy	Probe [†]	PB 7.4	654	n.d.*	670	~1,925
K8-1357	Square-680-NHS	NH ₂	MeOH	668	n.d.*	690	n.d.*
K8-1384	Seta-700-NHS	NH ₂	PB 7.4	687	n.d.*	703	n.d.*
K8-1389	Seta-695-NHS	NH ₂	PB 7.4	688	n.d.*	703	n.d.*
K8-1405	Square-650-pH-Carboxy	NH ₂	PB 5.6	653	n.d.*	671	n.d.*
K8-1407	Square-650-pH-NHS	NH ₂	PB 5.6	653	n.d.*	671	n.d.*
K8-1641	Seta-632-Maleimide	SH	PB 7.4	633	n.d.*	642	n.d.*

Product Number (Click for Specs Sheet)	Product Name (Click for Product Info)	Target Group [§]	Medium	$\lambda_{\max}$ Ab [nm]	Maximum 2PA Wavelength [nm]	$\lambda_{\max}$ FI [nm]	2P-Excitation Cross-Section [GM]
K8-1642	Seta-632-NHS	NH ₂	PB 7.4	632	n.d.*	641	n.d.*
K8-1663	Seta-633-NHS	NH ₂	PB 7.4	633	n.d.*	644	n.d.*
K8-1671	Seta-646-Maleimide	SH	PB 7.4	647	840	657	~480
K8-1672	Seta-646-NHS	NH ₂	PB 7.4	646	840	656	~480
K8-1682	Seta-660-di-NHS	NH ₂	PB 7.4	661	900	672	~1,625
K8-1696	Seta-633-Azide	triple-CC	PB 7.4	633	n.d.*	644	n.d.*
K9-3152	SeTau-488-NHS	NH ₂	PBS 7.4	486	n.d.*	532	n.d.*
K9-3153 ^{new}	SeTau-488-Maleimide	SH	PBS 7.4	484	n.d.*	531	n.d.*
K9-4119	SeTau-665-NHS	NH ₂	PB 7.4	664	n.d.*	712	n.d.*
K9-4142	SeTau-647-di-NHS	NH ₂	PB 7.4	650	n.d.*	694	n.d.*
K9-4145	SeTau-633-Ethyl-Ester	Probe [†]	CHCl ₃	634	900-920	683	>1,000
K9-4148	SeTau-647-Maleimide	SH	PB 7.4	648	n.d.*	692	~3,700
K9-4149	SeTau-647-NHS	NH ₂	PB 7.4	649	920	695	~3,700
K9-4150	SeTau-647	Probe [†]	PB 7.4	647	920	693	>3,000
K9-4154	SeTau-647-DBCO	N ₃	PB 7.4	649	920	690	~3,700
K9-4159 ^{new}	SeTau-660-NHS	NH ₂	PB 7.4	663	n.d.*	694	n.d.*
K9-4169	SeTau-670-NHS	NH ₂	PB 7.4	673	n.d.*	694	n.d.*
K9-4179	SeTau-680-NHS	NH ₂	PB 7.4	683	n.d.*	705	n.d.*

[§] Dyes with other target groups can be synthesized at your request.

* Not determined, but according to its chemical structure (substituted [squinine](#) / [squinine rotaxane](#) dye) this compound is expected to exhibit a high **2PACS**.

[†] Probe for non-covalent attachment.

Seta and **SeTau** dyes offer ideal fluorescence properties for in-vivo two-photon imaging. Importantly, SeTau dyes exhibit 2PACSs and photostabilities similar to quantum dots, but with much lower molecular weights, and therefore also allow the labelling of subcellular structures at low concentrations and with reduced functional impact. They are also non-toxic and are well-suited for long-term, neuronal imaging. The demonstrated brightness and stability of these dyes promise to extend the limits of fluorophore concentration, imaging rate, illumination depth, and imaging duration for in-vivo two-photon microscopy [1].

2-Photon FRET

2P-FRET microscopy has significant advantages over laser **confocal FRET microscopy**: while in **confocal FRET microscopy**, the actual FRET signal in the acceptor channel is always contaminated due to direct excitation of the acceptor molecule and the cross-talk between donor and acceptor emissions in the acceptor channel, in 2P-microscopy, it is possible to prevent the direct excitation of the acceptor molecule. 2P-microscopy also avoids out-of-focus bleaching, which allows repeated scanning in multiple focal planes and to obtain a more detailed image of intracellular events. **2P-FRET microscopy** is also a powerful tool to assay intracellular **protein-protein co-localization/interaction events**.

Below is some information on the **Förster radii**, **2P-excitation wavelengths**, and emission wavelengths of **2P-FRET pairs** that are based on a combination of our most efficient 2P-donors with several of our acceptor dyes:

2P-Donor (Specs Sheet / Product Info)	Acceptor (Specs Sheet / Product Info)	Förster Radii (Å)	2P-Excitation Wavelength [nm]	Emission Wavelengths [nm]	
				Donor	Acceptor
SeTau-405 / K7-547	Seta-555 / K8-3335	50–53	750–850	520	571
SeTau-405 / K7-547	Lyophilized R-PE / R-PE	78–85	750–850	520	573
SeTau-405 / K7-547	SQ-565 / K8-1902) [†]	41–45	750–850	520	— [†]
Seta-646 / K8-1672	Seta-670 / K8-1342	60–68	840	660	693
Seta-646 / K8-1672	SeTau-647 / K9-4142	71–76	840	660	695
Seta-646 / K8-1672	SeTau-647 / K9-4149	60–67	840	660	695
Seta-646 / K8-1672	Seta-750 / K8-7522	56–67	840	660	780
Seta-660 / K8-1682	Seta-750 / K8-7522	72–76	900	675	780
Seta-660 / K8-1682	SQ-740 / K8-1649 [†]	55–60	900	675	— [†]
SeTau-647 / K9-4149	SQ-740 / K8-1649 [†]	65	920	695	— [†]
SeTau-665 / K9-4119	SQ-740 / K8-1649 [†]	66	900	716	— [†]

[†] Quencher.

Reference

[1] Podgorski K. et al. Ultra-Bright and -Stable Red and Near-Infrared Squaraine Fluorophores for In Vivo Two-Photon Imaging. PLOS ONE 7(12): e51980 (2012).

Contact us about these and other products or if you are interested in obtaining **more specific information about a certain product.**