Fluorescence Polarization (FP) Labels

Dyes with short and long fluorescence lifetimes for polarization-based assays

Fluorescence polarization (FP) measurements are based on the assessment of the rotational mobility of intrinsically or extrinsically fluorescent molecules in solution. FP reflects the competition between the rotational motion of a fluorescent molecule and its fluorescence lifetime (FLT). It is a versatile, solution-based technique that has been widely used to study molecular interactions, enzymatic activity, and nucleic acid hybridization. Several reporter properties should be considered when developing FP assays, including the luminescence lifetime, linker length, hydrophobicity or hydrophilicity, quantum yield (QY), and extinction coefficient (ε) of the molecular reporter. The luminescent lifetime of the reporter, the size of the antigen, and the change in molecular weight upon binding determine the dynamic range of an FP assay.

SETA BioMedicals has developed fluorescent polarization-based reporters for a broad range of molecular-weights (MW ~ 1–80 kDa, see Table below) of analytes. Short-lifetime labels are generally suitable for small antigens, whereas long-lifetime SeTau labels extend the range of FP even to peptide/protein- or nucleotide-size antigens.

Product Number (Specs Sheet)	Product Name (Product Info)	Target Group	380	405	436	488	532	594	635	650	680	700	Medium	λ abs [nm]	ε [M –1 cm–1]	λ em [nm]	QY [%]	FLT [ns]
K1-204	SeTau-380-NHS	NH_2	•	•									Water	270	23,800	480	56	32.5
K4-205	Seta-375-NHS	NH_2	•										PB 7.4	372	24,500	476	86	3.1
K5-3222	Seta-580-NHS	NH_2					•						PB 7.4	578	90,000	602	55	n.d.*
K7-544	SeTau-425-Maleimide	SH	•	•	•								PB 7.4	425	4,200	545	39	26.2
K7-545	SeTau-425-NHS	NH_2	•	•	•								PB 7.4	425	4,200	545	39	26.2
K7-547	SeTau-405-NHS	NH_2	•	•									PB 7.4	405	13,800	518	80	9.3
K7-548	SeTau-405-Maleimide	SH	•	•									PB 7.4	405	13,800	518	51	9.1#
K7-567	SeTau-405-Azide	C≡C	•	•									PB 7.4	405	13,800	518	80	9.1#
K8-1341	Seta-670-Maleimide	SH	•	•					•	•			PB 7.4	667	180,000	688	7	0.42#
K8-1342	Seta-670-NHS	NH_2	•	•					•	•			PB 7.4	667	180,000	688	7	0.42#
K8-1346	Seta-670-Azide	C≡C	•	•					•	•			PB 7.4	667	180,000	686	7	0.42#
K8-1352	Square-660-NHS	NH_2	•	•					•	•			PB 7.4	657	182,000	676	3	0.27#
K8-1389	Seta-695-NHS	NH_2		•						•	•	•	PB 7.4	688	162,000	703	14	n.d.*
K8-1662	Seta-640-NHS	NH_2							•				PB 7.4	636	181,000	646	13	0.7
K8-1663	Seta-633-NHS	NH_2						•	•				PB 7.4	633	250,000	644	7	0.25
K8-1667	Seta-635-pH-di-NHS	NH_2							•				PB 7.4	640	188,000	656	33	1.8
K8-1671	Seta-646-Maleimide	SH							•				PB 7.4	647	210,000	657	8	n.d.*
K8-3002	Seta-470-NHS	NH_2		•	•	•							Water	469	50,000	521	1.9	0.21
K8-3003	Seta-470-Maleimide	SH		•	•	•							Water	469	50,000	521	1.9	n.d.*
K9-3152	SeTau-488-NHS	NH_2				•							PB 7.4	486	59,000	532	27	1.2#
K9-3153	SeTau-488-Maleimide	SH				•							PB 7.4	484	59,000	531	20	1.2#
K9-3162	SeTau-500-NHS	NH_2				•							PB 7.4	502	59,600	567	43	1.7
K9-4119	SeTau-665-NHS	NH_2								•	•		PB 7.4	664	160,000	712	53	3.1
K9-4142	SeTau-647-di-NHS	NH_2							•	•			PB 7.4	650	200,000	694	65	3.2
K9-4148	SeTau-647-Maleimide	SH							•	•			PB 7.4	648	200,000	692	45	3.2
K9-4149	SeTau-647-NHS	NH_2							•	•			PB 7.4	649	200,000	695	61	3.2
K9-4159	SeTau-660-NHS	NH_2							•	•			PB 7.4	663	240,000	694	50	3.36
K9-4169	SeTau-670-NHS	NH_2							•	•	•		PB 7.4	673	275,000	694	36	1.6
KS-370	330–405 nm FLT Standard	–§	•	•									PB 7.4	370	–	470	80	3.18
KS-405	370–420 nm FLT and FP Standard	–§	•	•									Water	405	–	518	–	9.07
KS-470	436–490 nm FP Standard	–§			•	•							PB 7.4	0.01	–	–	1.9	0.21
KS-550	510–560 nm FP Standard	–§					•						Water	549	–	567	–	n.d.*
KS-650	590–650 nm FLT Standard	–§											PB 7.4	647	250,000	665	27	0.98
KS-660	550–670 nm FP Standard	–§							•	•			PB 7.4	0.01	–	–	3	0.27

§ Dyes with other target groups can be synthesized at your request.
$ Contains carboxylic group that can be activated.
* Not determined.

SETA BioMedicals
Fluorescent Tools for BioMedical Applications

2014 Silver Ct. East, Urbana, IL 61801, USA
Email: support@setabiomedicals.com
Orders: orders@setabiomedicals.com
Phone: 1-217-417-2160

Short-Lifetime FP Labels

Short-lifetime Polarization Labels with lifetimes (τ) up to 3.2 ns are suitable for measurement of antigens with molecular weights approximately between 1 and 10 kDa.

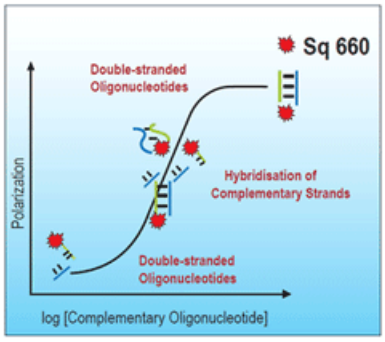
Measurement of DNA-oligonucleotide binding with Short-Lifetime Square-660 Label

DNA hybridization reactions can be readily monitored by fluorescence polarization (FP) using fluorescent labels developed by **SETA BioMedicals**. Square-660-NHS is a very sensitive near-infrared (NIR) fluorescent FP label that can be readily coupled to amino-modified oligonucleotides.

Covalent attachment of Square-660-NHS to an oligonucleotide increases its fluorescence lifetime from approximately 0.27 ns for the free dye to approximately 3 ns for the labeled oligonucleotide, resulting in a low initial polarization of the labeled probe. Hybridization of the dye-labeled oligonucleotide with a complementary oligonucleotide or with DNA increases the size and rotational correlation time of the resulting duplex.

Upon hybridization with the complementary strand, the Square-660 lifetime decreases from around 3 ns to around 1.8 ns. This reduction in τ, together with the increase in the rotational correlation time of the double-stranded oligo, produces a larger increase in fluorescence polarization than the weight increase of the duplex formation alone, thereby additionally expanding the dynamic range of the assay.

Due to the fluorescence lifetime increase upon attachment to oligonucleotides, its emission in the NIR range, and the additional polarization response observed upon hybridization, Square-660 is particularly suitable as a fluorescent reporter for homogeneous hybridization assays for small biomolecules using fluorescence polarization as the readout parameter.

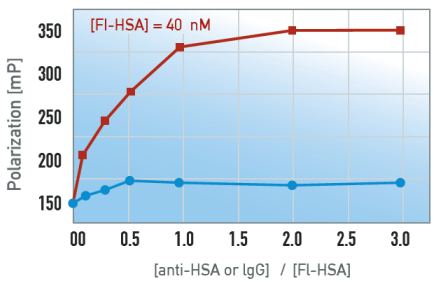


Long-Lifetime FP Labels

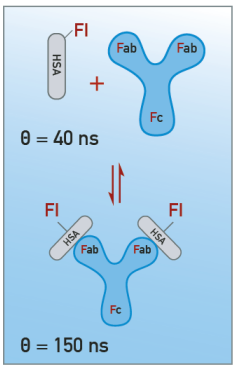
SeTau Fluorescence Polarization (FP) Labels exhibit fluorescence lifetimes (τ) of approximately 9–32 ns, which make them suitable for FP measurements of protein-, peptide- or nucleotide-based antigens in 10–80 kDa range. This range has not been covered by conventional short-lifetime organic fluorophores, though long-lifetime Ru, Os, and Re metal–ligand complexes have been used for high-molecular-weight fluorescence polarization immunoassay (FPIA) for a long time.

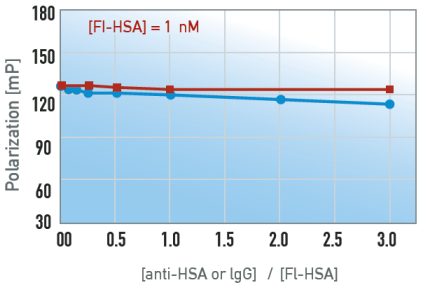
Comparison of Long-Lifetime of SeTau-425 and Short-Lifetime Fluorescein Labels in an FPIA of HSA

As an example, HSA (~66.5 kDa) was labeled either with SeTau-425 (τ = 26 ns; left figure below) or with fluorescein (τ = 4 ns; right figure below). The SeTau-425-labeled HSA showed a relatively low initial polarization of 165 mP. Upon addition of the specific antibody (brown curve, left panel), the rotational correlation time of the fluorescently (Fl) labeled antigen–antibody complex increased, resulting in a substantial polarization increase. In contrast, fluorescein-labeled HSA (right panel) showed an initial polarization of 130 mP that changed little upon addition of either specific (brown curve) or nonspecific (blue curve) antibody. The comparatively low initial value is consistent with homo-FRET between multiple fluorescein labels on the same HSA molecule. Moreover, because the fluorescein lifetime ($\tau = 4$ ns) is much shorter than the rotational correlation time of HSA (Θ = 40 ns), the additional increase in complex size upon antibody binding produces only a small change in polarization.



SeTau-425
τ = 26 ns





Fluorescein
τ = 4 ns

Contact us about these and other products.

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2014 Silver Ct. East, Urbana, IL 61801, USA
Email: support@setabiomedicals.com
Orders: orders@setabiomedicals.com
Phone: 1-217-417-2160