ATTO-TEC

Fluorescent Labels and Dyes **Revised: April 10, 2014**

Product Information: ATTO-Dye Labeled Streptavidin

Compound	Storage information	Shelf Life
Fluorescent streptavidin, solvent-free lyophilized solid	Freeze upon receipt ≤ - 20°C Protect from light and moisture	When stored as indicated, fluorescence labeled streptavidin is stable for at least 2 years.
For optical properties see Table 1 on page 2.		

Introduction:

Streptavidin, isolated from *Streptomyces avidinii*, is a tetrameric protein of 4 x 13.2 kDa and an extinction coefficient at 280 nm of ε_{280} = 167000 M^{-1} cm^{-1} [1]. Streptavidin binds very tightly to the small molecule biotin. The dissociation constant of the complex is extremely small ($K_d \approx 10^{-15}$ M), ranking among the strongest non-covalent interactions. This has made the streptavidin/biotin system a useful tool in numerous biochemical applications.

ATTO streptavidin conjugates may be used as secondary detection reagents in flow cytometry, immunoassays, blot analysis, histochemical applications, etc. The dye conjugates are supplied as solvent-free lyophilized solids. **ATTO streptavidin conjugates** are readily soluble in water. The available conjugates are shown in Table 1.

Storage and Handling:

ATTO-Dyes labeled streptavidines are supplied as lyophilisates and should be stored at ≤ –20°C, desiccated and protected from light. When stored as indicated, the product is stable for at least three years.

For the preparation of stock solutions allow vial to equilibrate to room temperature before opening. Dissolve the ATTO-streptavidin conjugate in distilled water to a concentration of 1 mg/ml. For long-term storage of such solutions one should add sodium azide to a concentration of 5 mM. Protected from light and stored at 2 - 6 °C, solutions are stable for up to six months. For longer storage you may divide the solution into aliquots and freeze at -20 °C. However, one should avoid repeated freezing-and-thawing cycles.

Labeling with ATTO-Dye Labeled Streptavidin:

We recommend to centrifuge protein conjugate solutions briefly before use (microcentrifuge). The supernatant will be free of protein aggregates that may have formed and could cause non-specific background binding. For most applications a streptavidin conjugate concentration of 1-10 µg/ml is satisfactory. However, staining protocols may vary considerably with the application at hand. Therefore one may need to determine the appropriate conjugate concentration empirically.

Table 1: Properties of ATTO-dye labeled streptavidin:

Dye	Order #		λ_{abs}	λ_{em}	ε_{max}	CF_{260}	CF_{280}
	1 mg	5 mg					
ATTO 390	AD 390-61	AD 390-65	390	465	24000	0.52	0.08
ATTO 425	AD 425-61	AD 425-65	445	477	45000	0.27	0.17
ATTO 430LS	AD 430LS-61	AD 430LS-65	440	536	32000	0.41	0.26
ATTO 465	AD 465-61	AD 465-65	460	504	75000	1.12	0.54
ATTO 488	AD 488-61	AD 488-65	503	525	90000	0.25	0.10
ATTO 490LS	AD 490LS-61	AD 490LS-65	502	649	40000	0.37	0.18
ATTO 514	AD 514-61	AD 514-65	514	533	115000	0.21	0.08
ATTO 532	AD 532-61	AD 532-65	537	555	115000	0.22	0.11
ATTO 540Q	AD 540Q-61	AD 540Q-65	547		105000	0.22	0.24
ATTO 550	AD 550-61	AD 550-65	556	575	120000	0.24	0.12
ATTO 565	AD 565-61	AD 565-65	568	593	120000	0.34	0.16
ATTO 590	AD 590-61	AD 590-65	601	623	120000	0.42	0.44
ATTO 594	AD 594-61	AD 594-65	608	633	120000	0.26	0.51
ATTO 610	AD 610-61	AD 610-65	624	640	150000	0.02	0.05
ATTO 612Q	AD 612Q-61	AD 612Q-65	621		115000	0.35	0.57
ATTO 620	AD 620-61	AD 620-65	625	644	120000	0.05	0.07
ATTO 633	AD 633-61	AD 633-65	638	657	130000	0.05	0.06
ATTO 647	AD 647-61	AD 647-65	648	671	120000	0.08	0.04
ATTO 647**N**	AD 647N-61	AD 647N-65	653	668	150000	0.06	0.05
ATTO 655	AD 655-61	AD 655-65	665	682	125000	0.24	0.08
ATTO 665	AD 665-61	AD 665-65	662	682	160000	0.07	0.06
ATTO 680	AD 680-61	AD 680-65	679	699	125000	0.30	0.17
ATTO 700	AD 700-61	AD 700-65	695	720	120000	0.26	0.41

λ_{abs}: longest-wavelength absorption maximum in nm (solvent: PBS, pH 7.4, degree of labeling (DOL): 2-3); λ_{em}: fluorescence maximum in nm (solvent: PBS, pH 7.4); ε_{max}: molar decadic extinction coefficient at the longest-wavelength absorption maximum in M^{-1} cm^{-1}; $CF_{260} = \varepsilon_{260}/\varepsilon_{max}$; $CF_{280} = \varepsilon_{280}/\varepsilon_{max}$;

[1] S.C. Gill, P.H. von Hippel, *Calculation of Protein Extinction Coefficients from Amino Acid Sequence Data*, Analytical Biochemistry **182**, 1989, 319-326.

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