Introduction:

Streptavidin, isolated from Streptomyces avidinii, is a tetrameric protein of 4 x 13.2 kDa with an extinction coefficient at 280 nm of ε280 = 167000 M^{-1} cm^{-1} [1]. Streptavidin binds very tightly to the small molecule biotin. The dissociation constant of the complex is extremely small (Kd ≈ 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. The available conjugates are shown in Table 1.

Storage and Handling:

ATTO-Dye labeled streptavidins are supplied as lyophilizates 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.
ATTO streptavidin conjugates are readily soluble in water. For the preparation of stock solutions allow the 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	λ abs	λ em	ε max	CF260	CF280
ATTO 390	390	465	24000	0.52	0.08
ATTO 425	445	477	45000	0.27	0.17
ATTO 430LS	440	536	32000	0.41	0.26
ATTO 465	460	504	75000	1.12	0.54
ATTO 488	503	525	90000	0.25	0.10
ATTO 490LS	502	649	40000	0.37	0.18
ATTO 514	514	555	115000	0.21	0.08
ATTO 532	537	555	115000	0.22	0.11
ATTO 540Q	547		105000	0.22	0.24
ATTO 542	542	562	120000	0.18	0.08
ATTO 550	556	575	120000	0.24	0.12
ATTO 565	568	593	120000	0.34	0.16
ATTO 590	601	623	120000	0.42	0.44
ATTO 594	608	633	120000	0.26	0.51
ATTO 610	624	640	150000	0.02	0.05
ATTO 612Q	621		115000	0.35	0.57
ATTO 620	625	644	120000	0.05	0.07
ATTO 633	638	657	130000	0.05	0.06
ATTO 643	643	665	150000	0.05	0.04
ATTO 647	648	671	120000	0.08	0.04
ATTO 647N	653	668	150000	0.06	0.05
ATTO 655	665	682	125000	0.24	0.08
ATTO 665	662	682	160000	0.07	0.06
ATTO 680	679	699	125000	0.30	0.17
ATTO 700	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 longestwavelength absorption maximum in M^{-1} cm^{-1}; CF260 = ε 260/ ε max; CF280 = ε 280/ ε 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.