ALPHA DIAGNOSTIC INTERNATIONAL

Product Data Sheet

Streptavidin-Pe-Cy5.5 and Cy7 Conjugates

☐ **Cat#.** SV-PEC5-100	Streptavidin-PE-Cy5.5 conjugate	**Size:**	100 tests
☐ **Cat#.** SV-PEC7-100	Streptavidin-PE-Cy7 conjugate	**Size:**	100 tests

Streptavidin is a 53 Kda tetrameric protein purified from the bacterium Streptomyces avidinii. It finds wide uses in immunhoassay and molecular biology due to its extraordinarily strong affinity for the vitamin biotin; the dissociation constant (Kd) of the biotin-streptavidin complex is on the order of ~10-15 mol/L, ranking among one of the strongest known non-covalent interactions. There are considerable differences in the composition of avidin (found in egg white) and streptavidin, but they are remarkably similar in other respects. Both proteins form tetrameric complexes to function in which each subunit can bind one molecule of biotin. Streptavidin is much less soluble in water than avidin, and it lacks avidin's extensive glycosylation. Streptavidin has a mildly acidic isoelectric point (pI) of ~5. Because streptavidin lacks any carbohydrate modification and has a near-neutral pI, it has the advantage of much lower nonspecific binding than avidin. Deglycosylated avidin is more comparable to the size, pI and nonspecific binding of streptavidin.

Streptavidin's affinity for biotin is exploited in wide ranging biochemical assays, including western blot, ELISA, ELISPOT and pull-down assays. Streptavidin immobilized onto solid supports (ELISA plates, agarose, nitrocellulose etc) is also used as purification media to capture biotin-labelled protein or nucleic acid molecules. For example, cell surface proteins can be specifically labelled with membrane impermeable biotin reagent, then specifically captured using an avidin-based support.

Purified streptavidin is available as HRP, AP, FITC, Rhodamine, and phycoerythrin (PE), PE-TR conjugate.

Cat# SV-PEC5-100, PE-Cy5.5 conjugate

Purified Streptavidin was coupled to PE-Cy5.5 (Cyanine 5.29-OSu) (Molecular Weight 975 daltons) at F/P ratio ~6:1. The antibody is supplied in PBS, pH 7.4, 0.5% BSA, IgG, and 0.05% azide in either **lyophilized** or **liquid** form (see lot specific conc on the vial). Reconstitute powder in PBS. Store at -20oC in suitable aliquots. Stability is ~6-12 months. Do not freeze and thaw.

Suggested applications: Suitable for immunomicroscopy and flow cytometry or FACS analysis as well as other antibody based fluorescent assays

Suggested conjugate dilutions are 2-5 ul/tests for immunofluorescence. Users must optimize the dilutions for a given technique. Or 1 test equivalent of antibody per million cells.

Absorption Wavelength: 650 nm
Emission Wavelength: 667 nm

Cat# SV-PEC7-100, PE-Cy7 conjugate

Purified Streptavidin was coupled to PE-Cy7

1. This reagent has been pre-diluted for use at the recommended Volume per Test. **We recommend 5 ul/test** (use 1 X 10^6 cells in a 100-µl experimental sample or a test). **Note**: Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. PE-Cy7 is a tandem fluorochrome composed of R-phycoerythrin (PE), which is excited by the 488-nm line of a laser and serves as an energy donor, coupled to the cyanine dye Cy7., which acts as an energy acceptor and fluoresces at 780 nm. The product has maximal fluorochrome energy transfer in PE-Cy7, thus maximizing its fluorescence emission intensity and minimizing residual emission from PE. **Note**: The lot-to-lot variation in residual emission from PE is minimized, it is strongly recommended that every lot be tested for differences in the amount of compensation required and that individual compensation controls are run for each PE-Cy7 conjugate.
3. PE-Cy7 tandem fluorochrome emission is collected in a detector for fluorescence wavelengths of 750 nm and higher. PE-Cy7-labeled antibodies can be used with FITC- and R-PE-labeled reagents in single-laser flow cytometers with no significant spectral overlap between PE-Cy7 and FITC.
4. PE-Cy7 is optimized for use with a single argon ion laser emitting 488-nm light. Because of the broad absorption spectrum of the PE-Cy7 tandem fluorochrome, extra care must be taken when using dual-laser cytometers which may directly excite both PE and Cy7.. We recommend the use of cross-beam compensation during data acquisition or software compensation during data analysis.
5. Please observe the following precautions: Absorption of visible light can significantly alter the energy transfer occurring in any tandem fluorochrome conjugate; therefore, we recommend that special precautions be taken (such as wrapping vials, tubes, or racks in aluminum foil) to prevent exposure of conjugated reagents, including cells stained with those reagents, to room illumination.
6. Cy. is a trademark of Amersham Biosciences Limited. This product is subject to proprietary rights of Amersham Biosciences Corp. and Carnegie Mellon University.

SV-PEC5-100 140326A

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