Biotin-Streptavidin Conjugation

Streptavidin is a tetrameric biotin-binding protein derived from the bacterium *Streptomyces avidinii*. Streptavidin binds up to four biotin molecules with high affinity and selectivity via multiple hydrogen bonds and van der Waals interactions. Due to the lack of carbohydrate modifications and a near-neutral pI, streptavidin exhibits less nonspecific binding than another biotin-binding protein — avidin. Streptavidin also has high thermostability and resistance against extreme pH, denaturing agents, and enzymatic degradation, allowing using this protein under various experimental conditions.

Fluorescent conjugates of streptavidin are commonly used as a second-step reagent for specific detection of a variety of biotin-labeled biomolecules, such as proteins (antibodies, etc.), nucleic acids, lipids, and other molecules in indirect immunofluorescent staining, western blots, flow cytometry, microplate assays, and other detection techniques.

Lumiprobe provides a line of fluorescently labeled streptavidin products covering all the most widely used wavelengths for microscopy and flow cytometry:

Catalog Number	Fluorophore	Excitation, nm	Emission, nm
x1AS0	AMCA	348	435
x18S0	AF 488	495	519
x68S0	AF 594	586	613
x13S0	sulfo-Cyanine3	548	563
x33S0	sulfo-Cyanine5	646	662

Applications

- Streptavidin conjugates are used as a second-step reagent in histo- and cytochemistry, flow cytometry, blot analysis, and immunoassays.
- Streptavidin conjugates can also be used to localize biocytin, biocytin-X, biotin ethylenediamine, and fluorescently labeled biocytins — derivatives of biotin that are widely used as neuroanatomical tracers.
- Streptavidin conjugates can be pre-mixed with biotin to make a streptavidin-biotin complex and perform streptavidin-biotin (sABC) signal amplification.

Stock solution preparation and handling

- Streptavidin conjugates are supplied freeze-dried in 0.1 M phosphate buffered saline (PBS), pH 7.4.
- Reconstitute 100 µg of streptavidin conjugate powder (A1AS0, A18S0, A68S0, A13S0, A33S0) with 100 µL distilled or deionized water to obtain 1 mg/mL stock solution.
- Reconstitute 1 mg of streptavidin conjugate powder (11AS0, 118S0, 168S0, 113S0, 133S0) with 1 mL distilled or deionized water to obtain 1 mg/mL stock solution.

Lumiprobe Corporation
201 International Circle, Suite 135
Hunt Valley, Maryland 21030
USA
Phone: +1 888 973 6353
Fax: +1 888 973 6354
Email: order@lumiprobe.com

Lumiprobe GmbH
Feodor-Lynen-Strasse 23
30625 Hannover
Germany
Phone: +49 511 16596811
Fax: +49 511 16596815
Email: de@lumiprobe.com

Lumiprobe RUS Ltd
Kotsyubinsky street, 4
121351 Moscow
Russian Federation
Phone: +7 800 775 3271
Email: ru@lumiprobe.com

- Reconstituted streptavidin solutions are stable for approximately six months with the addition of sodium azide to a final concentration of 5 mM or thimerosal to 0.2 mM.
- For extended storage, divide solutions into aliquots and freeze at <-20 °C. Protect from light. Avoid repeated freezing and thawing of solutions

Protocol

Before you start

- Avoid using biotin-containing solutions (some serums, RPMI 1640, etc.) as diluents.
- Centrifuge protein (antibody or streptavidin) solutions briefly in a microcentrifuge before use and take only the supernatant for the experiment. This step eliminates any protein aggregates that may have formed in the solution, thereby reducing nonspecific background staining.
- Perform a titration to determine the best concentration of antibody and streptavidin to use. Start with a fixed amount of the biotinylated antibody, using the recommended dilution from the supplier, and titrate the fluorescently labeled streptavidin. If the high nonspecific staining is still observed, titrate the primary antibody using the fixed amount of the fluorescently labeled streptavidin that has been determined.
- Although streptavidin gives negligible background staining, it is recommended to perform the step of blocking nonspecific antibody binding sites that is in most immunochemistry protocols.
- For membrane blot applications, Tween® 20 (0.1 to 0.2% v/v) and SDS (0.02% to 0.1% v/v) should be included in the detection buffer for the final incubation step to minimize nonspecific background staining.

The following are commonly used methods for employing streptavidin as a secondary detection reagent.

Indirect method

The most common method for using labeled streptavidin is a two-step staining protocol. The first step involves introducing a biotinylated probe into a cell, tissue, or surface. The second step involves binding fluorescently labeled streptavidin to the probe's biotin.

1. Incubate cells or tissue with a biotinylated probe, such as an antibody, single-stranded nucleic acid probe, or lectin, according to the probe's manufacturer's recommendations.
2. Wash cells/tissue with PBS or another suitable buffer to remove excess biotinylated probe.
3. Dilute the streptavidin conjugate stock solution (1 mg/mL) with buffer to prepare the working streptavidin solution. A final concentration of 0.5–10 µg/mL is usually satisfactory for most histo- and cytochemical applications. Because staining protocols vary with the application, determine appropriate dilutions of conjugates empirically.
4. Incubate cells with fluorescently labeled streptavidin for 60 minutes at room temperature.
5. Wash cells/tissue several times with buffer to remove excess streptavidin.

Lumiprobe Corporation
201 International Circle, Suite 135
Hunt Valey, Maryland 21030
USA
Phone: +1 888 973 6353
Fax: +1 888 973 6354
Email: order@lumiprobe.com

Lumiprobe GmbH
Feodor-Lynen-Strasse 23
30625 Hannover
Germany
Phone: +49 511 16596811
Fax: +49 511 16596815
Email: de@lumiprobe.com

Lumiprobe RUS Ltd
Kotsyubinsky street, 4
121351 Moscow
Russian Federation
Phone: +7 800 775 3271
Email: ru@lumiprobe.com

Direct method

An alternative method is pre-mixing biotinylated antibodies or other biotin-containing molecules with fluorescently labeled streptavidin prior to incubation with cells or tissue. A direct method can be required when more than one biotinylated molecule is used in a multiplexing panel. In this case, each biotinylated molecule should be pre-mixed with a different fluorescently labeled streptavidin.

Important! *There may be rare cases when the direct method doesn't work. If an excess quantity of biotins is attached to an antibody or biotin residues are too close to the antigen binding site, there will be steric hindrance, resulting in loss of binding. In these cases, an indirect method will be preferable for good staining.*

1. Mix a biotinylated probe such as an antibody, single-stranded nucleic acid probe, or lectin with fluorescently labeled streptavidin and let it stand for about 30 minutes at room temperature before use. The pre-mix is stable for at least 7 days and can be used for a week after preparation.

   This method requires optimization of the ratio of the biotinylated probe to fluorescently labeled streptavidin. Recommended ratios for initial testing are 3:1, 1:1, and 1:3 of probe to streptavidin. Optimization of the ratio only needs to be done once for each biotinylated probe-streptavidin pair.
2. Incubate cells or tissue with a pre-mixed probe-streptavidin complex for 60 minutes or longer at room temperature.
3. Wash cells/tissue several times with buffer to remove excess streptavidin.

Lumiprobe Corporation
201 International Circle, Suite 135
Hunt Valey, Maryland 21030
USA
Phone: +1 888 973 6353
Fax: +1 888 973 6354
Email: order@lumiprobe.com

Lumiprobe GmbH
Feodor-Lynen-Strasse 23
30625 Hannover
Germany
Phone: +49 511 16596811
Fax: +49 511 16596815
Email: de@lumiprobe.com

Lumiprobe RUS Ltd
Kotsyubinsky street, 4
121351 Moscow
Russian Federation
Phone: +7 800 775 3271
Email: ru@lumiprobe.com