

Avidin and NeutrAvidin™ Biotin-Binding Proteins and Conjugates

Table 1. Contents and storage information.

| Material | Amount | Concentration | Storage Upon Receipt | Stability |
|--|--------------------------|---|--|-----------|
| Unlabeled avidin | 5 mg (A2667) | Lyophilized powder | <ul style="list-style-type: none"> • ≤-20°C • Desiccate | 3 years |
| | 100 mg (A887) | | | |
| Unlabeled NeutrAvidin™ | 5 mg (A2666) | | | |
| Fluorescent avidin conjugates | 5 mg (A820, A821, A2641) | Lyophilized powder | <ul style="list-style-type: none"> • ≤-20°C • Desiccate • Protect from light | 2 years |
| | 1 mg (A21370) | | | |
| Fluorescent NeutrAvidin™ conjugates | 1 mg | | | |
| R-Phycoerythrin conjugate of NeutrAvidin™ Biotin-Binding Protein | 1 mL (A2660) | 1 mg/mL solution in 0.1 M sodium phosphate, 0.1 M NaCl, 5 mM sodium azide, pH 7.5 | <ul style="list-style-type: none"> • 2–6°C • Protect from light • Do not freeze | 6 months |

Approximate fluorescence excitation/emission maxima: See Table 2.

Introduction

Invitrogen provides a full line of labeled and unlabeled avidin products (Table 2), including conjugates of the traditional avidin and of NeutrAvidin™ biotin-binding protein. The high affinity of avidin for biotin was first exploited in histochemical applications in the mid-1970s.^{1,2} This egg-white protein and its bacterial counterpart, streptavidin, have since become standard reagents for diverse detection schemes.³ In their simplest form, such methods entail applying a biotinylated probe to the sample and then detecting the bound probe with labeled avidin, streptavidin, or NeutrAvidin™ biotin-binding protein. These techniques are commonly used to localize antigens in cells and tissues^{4,5} and to detect biomolecules in immunoassays and DNA hybridization techniques.⁶⁻⁹

Avidin is a positively charged 66,000-dalton glycoprotein¹⁰ with an isoelectric point of about 10.5. It is thought that avidin's positively charged residues and its oligosaccharide component (heterogeneous structures composed largely of mannose and *N*-acetylglucosamine) can interact nonspecifically with negatively charged cell surfaces and nucleic acids, sometimes causing background problems in histochemical applications and flow cytometry. Methods have been developed to suppress this nonspecific avidin binding.¹¹ Avidin and its conjugates selectively bind to a component in mast cell granules, and can be used to identify mast cells in normal and diseased human tissue.^{12,13}

Our conjugates of NeutrAvidin™ biotin-binding protein—a 60,000-dalton protein that has been processed to remove the carbohydrate and lower its isoelectric point (6.3)—can sometimes reduce background staining. The methods used to deglycosylate the avidin are reported to retain its specific binding and its complement of amine-conjugation sites. NeutrAvidin™ conjugates have been shown to provide improved detection of single-copy genes in metaphase chromosome spreads.¹⁴

Avidin and NeutrAvidin™ biotin-binding protein each bind four biotins per molecule with high affinity and selectivity. Their multiple binding sites permit a number of techniques in which unlabeled avidin or NeutrAvidin™ biotin-binding protein can be used to bridge two biotinylated reagents. This bridging method, which is commonly used to link a biotinylated probe to a biotinylated enzyme in enzyme-linked immunohistochemical applications, often eliminates the background problems that can occur when using direct avidin- or streptavidin-enzyme conjugates.

In addition to the products listed in this product information sheet, Invitrogen prepares conjugates of streptavidin, as well as many species-specific anti-IgG antibodies and conjugates of protein A and protein G. Visit our web site (www.probes.com) for additional information.

Specific Activity

The specific activity avidin and NeutrAvidin™ is ~13 U/mg, where one unit of avidin is defined as the amount of protein required to bind 1 µg of biotin.

Table 2. Unlabeled streptavidin and fluorescent-dye conjugates of streptavidin.

| Label | Ex* | Em* | Avidin | NeutrAvidin™ |
|----------------------------|--------------------|-----|----------------|--------------|
| Unlabeled | NA | NA | A887, A2667 | A2666 |
| Fluorescent Dye Conjugates | | | | |
| Alexa Fluor® 350 | 346 | 442 | – | A11236 |
| Marina Blue™ | 365 | 460 | – | A11230 |
| Cascade Blue® | 400 | 420 | – | A2663 |
| Pacific Blue™ | 410 | 455 | – | A11231 |
| Fluorescein | 494 | 518 | A821 | A2662 |
| Alexa Fluor® 488 | 495 | 519 | A21370 | – |
| Oregon Green® 488 | 496 | 524 | – | A6374 |
| R-Phycoerythrin | 496, 546, 565 † | 578 | – | A2660 |
| BODIPY® FL | 505 | 513 | A2641 | A2661 |
| Tetramethylrhodamine | 555 | 580 | – | A6373 |
| Rhodamine Red™-X | 570 | 590 | – | A6378 |
| Texas Red® | 595 | 615 | A820 | A2665 |

*Approximate fluorescence excitation (Ex) and emission (Em) maxima for conjugates, in nm. Complete spectra for most of these dyes are available at our website. †Multiple excitation peaks. NA = Not applicable.

Before Starting

Preparing Solutions

Unlabeled conjugates

Prepare unlabeled avidin solutions of up to 10 mg/mL by dissolving the powder in phosphate-buffered saline (PBS) or other suitable buffer.

Prepare unlabeled NeutrAvidin™ biotin-binding protein solution by dissolving the contents of the vial (5 mg) in 1.0 mL PBS or other suitable buffer to obtain a 5 mg/mL solution.

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

Fluorescent conjugates

Prepare fluorescent avidin conjugate solutions of 2 mg/mL by dissolving the powder in the appropriate amount of PBS or other suitable buffer.

Prepare fluorescent NeutrAvidin™ conjugate solutions of 1 mg/mL by dissolving the powder in 1.0 mL PBS or other suitable buffer.

Reconstituted 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 longer storage, divide solutions into aliquots and freeze at -20°C . **Protect from light, and avoid repeated freezing and thawing of solutions.**

The approximate degree of labeling of the conjugate is listed on the product label.

Applications

Avidin and NeutrAvidin™ conjugates are used as secondary detection reagents in histochemical applications, flow cytometry,^{15,16} blot analysis, and immunoassays. These reagents can also be employed to localize biocytin, biocytin-X, biotin ethylenediamine and Alexa Fluor®, Cascade Blue® or lucifer yellow biocytins—derivatives of biotin that are used as neuroanatomical tracers.^{17,18}

The following are commonly used methods for employing avidin and NeutrAvidin biotin-binding protein as secondary detection reagents.

Direct Procedure

A biotinylated primary probe such as an antibody, single-stranded nucleic acid probe or lectin is bound to tissues, cells or other surfaces. Excess protein is removed by washing, and detection is mediated by reagents such as our fluorescent avidins or NeutrAvidin™ biotin-binding protein.

Indirect Procedure

A biotinylated antibody or oligonucleotide is used to probe a tissue, cell, or other surface.

This preparation is then treated with unlabeled avidin or NeutrAvidin™ biotin-binding protein. Excess reagents are removed by washing, and detection is mediated by a biotinylated detection reagent such as fluorescein biotin (Cat. no. B1370), biotinylated R-phycoerythrin

(Cat. no. P811), biotinylated FluoSpheres® microspheres or a biotinylated enzyme (Cat. no. P917) plus a substrate.

Alternatively, an unlabeled primary antibody is bound to a cell followed by a biotinylated species-specific secondary antibody. After washing, the complex is detected by the direct or indirect procedures described above.

Before use, centrifuge protein conjugate solutions briefly in a microcentrifuge and use only the supernatant for the experiment. This step eliminates any protein aggregates that may have formed in solution, thereby reducing nonspecific background staining. Because staining protocols vary with the application, empirically determine appropriate dilutions of conjugates. For fluorescent dye conjugates of avidins, including the R-phycoerythrin conjugate, a final concentration of 1–10 µg/mL is usually satisfactory for most histochemical applications.

References

1. Proc Natl Acad Sci USA 71, 3537 (1974); **2.** Biochim Biophys Acta 264, 165 (1972); **3.** Methods Enzymol 184 (1990); **4.** J Cell Biol 111, 1183 (1990); **5.** Physiol Plantarum 79, 231 (1990); **6.** Cytometry 11, 126 (1990); **7.** Proc Natl Acad Sci USA 87, 6223 (1990); **8.** Science 249, 928 (1990); **9.** Anal Biochem 171, 1 (1988); **10.** Adv Protein Chem 29, 85 (1975); **11.** J Histochem Cytochem 29, 1196 (1981); **12.** J Histochem Cytochem 33, 27 (1985); **13.** Invest Dermatol 83, 214 (1984); **14.** Trends Genet 9, 71 (1993); **15.** J Microbial Methods 12,1 (1990); **16.** Biochemistry 16, 5150 (1977); **17.** J Neurosci 10, 3421 (1990); **18.** Brain Res 497, 361 (1989).

Product List Current prices may be obtained from our website or from our Customer Service Department.

| Cat. no. | Product Name | Unit Size |
|----------|---|-----------|
| A21370 | avidin, Alexa Fluor® 488 conjugate | 1 mg |
| A2641 | avidin, BODIPY® FL conjugate | 5 mg |
| A2667 | avidin, egg white | 5 mg |
| A887 | avidin, egg white | 100 mg |
| A821 | avidin, fluorescein conjugate | 5 mg |
| A2666 | avidin, NeutrAvidin™ biotin-binding protein | 5 mg |
| A11236 | avidin, NeutrAvidin™, Alexa Fluor® 350 conjugate | 1 mg |
| A2661 | avidin, NeutrAvidin™, BODIPY® FL conjugate | 1 mg |
| A2663 | avidin, NeutrAvidin™, Cascade Blue® conjugate | 1 mg |
| A2662 | avidin, NeutrAvidin™, fluorescein conjugate | 1 mg |
| A11230 | avidin, NeutrAvidin™, Marina Blue® conjugate | 1 mg |
| A6374 | avidin, NeutrAvidin™, Oregon Green® 488 conjugate | 1 mg |
| A11231 | avidin, NeutrAvidin™, Pacific Blue™ conjugate | 1 mg |
| A6378 | avidin, NeutrAvidin™, Rhodamine Red™-X conjugate | 1 mg |
| A2660 | avidin, NeutrAvidin™, R-phycoerythrin conjugate *1 mg/mL* | 1 mL |
| A6373 | avidin, NeutrAvidin™, tetramethylrhodamine conjugate | 1 mg |
| A2665 | avidin, NeutrAvidin™, Texas Red® conjugate | 1 mg |
| A820 | avidin, Texas Red® conjugate | 5 mg |

Contact Information

Molecular Probes, Inc.

29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:

6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:

Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:

8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters

Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

**For country-specific contact information,
visit www.invitrogen.com.**

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