7.6 Avidin, Streptavidin, NeutrAvidin and CaptAvidin Biotin-Binding Proteins and Affinity Matrices

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.[3,4] In their simplest form, such methods entail applying a biotinylated probe to the sample and then detecting the bound probe with a labeled avidin or streptavidin. These techniques are commonly used to localize antigens in cells and tissues [5,6] and to detect biomolecules in immunoassays and DNA hybridization techniques [7–10] (Section 8.5). In addition to our important dye and enzyme conjugates of avidins and streptavidins, this section contains several products that can be used for the affinity isolation of biotin- and DSB-X biotin–conjugated molecules and their complexes with targets in cell and tissues. Our unique DSB-X biotin technology, which is described below, provides the most facile means available for reversing the strong interaction of biotin derivatives with avidins. The price lists in Chapter 4 contain complete lists of all of our biotin-containing probes, including biotinylation reagents, biotin-based tracers and biotinylated site-selective probes, as well as our important DSB-X biotin reagents and conjugates.

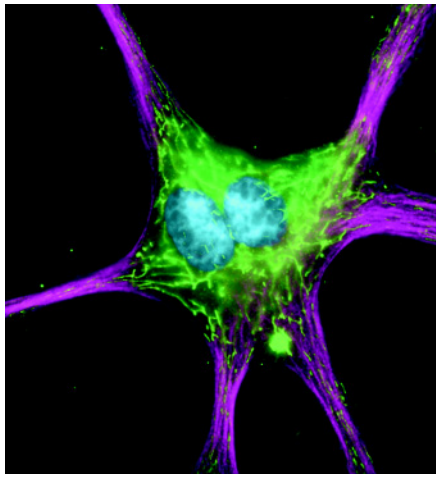

Figure 7.84 The cytoskeleton of a fixed and permeabilized bovine pulmonary artery endothelial cell detected using mouse monoclonal anti–α-tubulin antibody (A-11126), visualized with Alexa Fluor 647 goat anti–mouse IgG antibody (A-21235) and pseudocolored magenta. Endogenous biotin in the mitochondria was labeled with green-fluorescent Alexa Fluor 488 streptavidin (S-11223) and DNA was stained with blue-fluorescent DAPI (D-1306, D-3571, D-21490).

Binding Characteristics of Biotin-Binding Proteins

Avidin, streptavidin and NeutrAvidin biotin-binding protein each bind four biotins per molecule with high affinity and selectivity. Dissociation of biotin from streptavidin (S-888) is reported to be about 30 times faster that dissociation of biotin from avidin [11] (A-887, A-2667). Their multiple binding sites permit a number of techniques in which unlabeled avidin, streptavidin 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. However, a few endogenously biotinylated proteins that have carboxylase activity are found in the mitochondria (Figure 7.84, Figure 12.28); therefore, sensitive detection of biotinylated targets in cells requires the use of biotin-blocking agents to reduce this background.[12,13] Our Endogenous Biotin Blocking Kit (E-21390, see below) provides the reagents and a protocol for this application. Nonspecific binding of avidin conjugates of enzymes to nitrocellulose can be blocked more effectively by adding extra salts to buffers rather than by adding protein-based blocking reagents.[14]

High-purity unlabeled avidin (A-887), streptavidin (S-888), NeutrAvidin biotin-binding protein (A-2666) and CaptAvidin biotin-binding protein (C-21385) are available in bulk from Molecular Probes at reasonable prices. We also offer avidin specially packaged in a smaller unit size for extra convenience (A-2667). Each of our avidin, streptavidin and deglycosylated NeutrAvidin biotin-binding protein bind greater than 12 µg of biotin per mg protein. See below for a description of reversible binding of biotinylated targets with our CaptAvidin biotin-binding protein and other affinity matrices.

Avidin

Avidin (A-887, A-2667; Table 7.17) is a highly cationic 66,000-dalton glycoprotein [15,16] 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 some histochemical applications and flow cytometry. Methods have been developed to suppress this nonspecific avidin binding.[13] In some cases it can also be exploited. For example, avidin and its conjugates selectively bind to a component in rodent and human mast cell granules in fixed-cell preparations and can be used to identify mast cells in normal and diseased human tissue without requiring a biotinylated probe.[17,18]

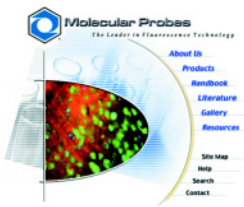

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Table 7.17 Molecular Probes' selection of avidin, streptavidin, NeutrAvidin and CaptAvidin conjugates.

Label (Abs/Em Maxima) *	Streptavidin	NeutrAvidin	Avidin	CaptAvidin
Fluorescent conjugates				
Alexa Fluor 350 (346/442)	S-11249	A-11236		
Marina Blue (365/460)	S-11221	A-11230		
Cascade Blue (400/420)		A-2663		
Pacific Blue (410/455)	S-11222			
Alexa Fluor 430 (431/541)	S-11237			
Fluorescein (494/518)	S-869	A-2662	A-821	
Alexa Fluor 488 (495/519)	S-11223		A-21370	
Oregon Green 488 (496/524)	S-6368	A-6374		
FluoSpheres (505/515)	F-8780	F-8771		
Oregon Green 514 (511/530)	S-6369			
Alexa Fluor 532 (530/554)	S-11224			
Alexa Fluor 546 (556/575)	S-11225			
Alexa Fluor 555 (555/565)	S-21381			
Tetramethylrhodamine (555/580)	S-870	A-6373		
R-Phycoerythrin (496/578)	S-866	A-2660		
Rhodamine B (570/590)	S-871			
Rhodamine Red-X (570/590)	S-6366	A-6378		
Alexa Fluor 568 (578/603)	S-11226			
Alexa Fluor 594 (590/617)	S-11227			
Texas Red (595/615)	S-872	A-2665	A-820	
Texas Red-X (595/615)	S-6370			
Alexa Fluor 610–R-PE (496/630)	S-20982			
Alexa Fluor 633 (632/647)	S-21375			
Alexa Fluor 647 (650/668)	S-21374			
Alexa Fluor 647–R-PE (496/668)	S-20992			
Allophycocyanin (650/660)	S-868			
Alexa Fluor 660 (663/690)	S-21377			
Alexa Fluor 680 (679/702)	S-21378			
Alexa Fluor 680–R-PE (496/702)	S-20985			
Alexa Fluor 680–allophycocyanin (650/702)	S-21002			
Alexa Fluor 700–allophycocyanin (650/723)	S-21005			
Alexa Fluor 750–allophycocyanin (650/775)	S-21008			
Alexa Fluor 700 (702/723)	S-21383			
Alexa Fluor 750 (749/775)	S-21384			
Other conjugates				
Agarose	S-951			C-21386
Alkaline phosphatase	S-921			
β-Galactosidase	S-931			
Horseradish peroxidase	S-911	A-2664		
NANOGOLD	N-24918			
Alexa Fluor FluoroNanogold	A-24926, A-24927			
CMNB-caged fluorescein	S-21380			
Acrylamide	S-21379			C-21387
Unlabeled avidins				
Unlabeled	S-888	A-2666	A-887, A-2667 †	C-21385

***** Approximate absorption (Abs) and fluorescence emission (Em) maxima, in nm, for conjugates. **†** Special packaging.

Streptavidin

Streptavidin (S-888, Table 7.17), a nonglycosylated 52,800-dalton protein with a near-neutral isoelectric point, reportedly exhibits less nonspecific binding than avidin. However, streptavidin contains the tripeptide sequence Arg–Tyr–Asp (RYD) that apparently mimics the Arg–Gly–Asp (RGD) binding sequence of fibronectin, a component of the extracellular matrix that specifically promotes cellular adhesion.[19] This universal recognition sequence binds integrins and related cell-surface molecules.[20,21] Background problems sometimes associated with streptavidin may be attributable to this tripeptide. We have particularly observed binding of streptavidin and anti-biotin [22] conjugates to mitochondria in some cells (Figure 7.84, Figure 12.28) that can be blocked with the reagents in our Endogenous Biotin Blocking Kit (E-21390, see below).

NeutrAvidin Biotin-Binding Protein

Molecular Probes provides an alternative to the commonly used avidin and streptavidin. Our conjugates of NeutrAvidin biotin-binding protein (A-2666, Table 7.17) — a protein that has been processed to remove the carbohydrate and lower its isoelectric point — can sometimes reduce background staining. The methods used to deglycosylate the avidin are reported to retain both its specific binding [23] and its complement of amine-conjugation sites. NeutrAvidin conjugates have been shown to provide improved detection of single-copy genes in metaphase chromosome spreads.[24]

CaptAvidin Biotin-Binding Protein: Reversible Binding of Biotinylated Molecules

CaptAvidin biotin-binding protein is our newest avidin derivative (C-21385, Table 7.17). Selective nitration of tyrosine residues in the four biotin-binding sites of avidin considerably reduces the affinity of the protein for biotinylated molecules above pH 9. Consequently, biotinylated probes can be adsorbed at neutral pH and released at pH ~10 (Figure 7.85). We use free biotin to block any remaining high-affinity biotin-binding sites that have not been nitrated. CaptAvidin agarose (C-21386, see below) is particularly useful for separation and purification of biotin conjugates from complex mixtures. The biotin-binding capacity of CaptAvidin derivatives is at least 10 µg biotin per mg protein.

Secondary Detection with Avidins

Avidin, streptavidin and NeutrAvidin conjugates are extensively used as secondary detection reagents in histochemical applications (Figure 7.86, Figure 7.87), FISH (Section 8.5, Figure 8.83), flow cytometry,[25,26] microarrays (Section 8.5, Figure 6.39), blot analysis (Section 9.4, Figure 9.58) and immunoassays. These reagents can also be employed to localize biocytin, biocytin-X, biotin ethylenediamine or one of our fluorescent biocytins — all of which are biotin derivatives commonly used as neuroanatomical tracers [27,28] (Section 14.3). DSB-X desthiobiocytin (D-20652, Section 14.3) is a similar polar

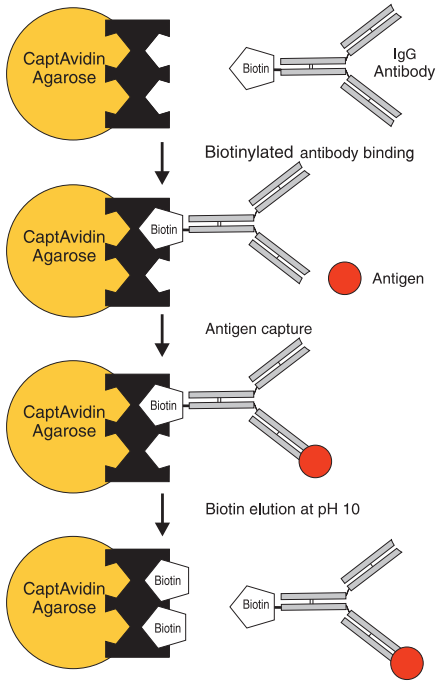


Figure 7.85 Diagram of the use of CaptAvidin agarose in affinity chromatography. A biotinylated IgG molecule and target antigen are used as an example.

Reversible Binding To Avidins

Molecular Probes exclusively supplies two important new technologies for the fully reversible capture of labeled probes — CaptAvidin biotin-binding protein and our DSB-X biotin–labeled reagents and purification kits. CaptAvidin binding of biotin can be reversed by raising the pH of the surrounding solution above pH 9, whereas the avidin–DSB-X biotin interaction can be reversed in physiological buffers by the addition of free biotin. When used with our Captivate ferrofluid streptavidin (C-21476), DSB-X biotin–labeled reagents can be used for the magnetic capture and subsequent release of rare viable cells from complex fluids. The Captivate microscope-mounted yoke assembly (C-24700, Figure 7.62) can be used in conjunction with the Captivate ferrofluid conjugates to separate cells and prepare them for imaging.

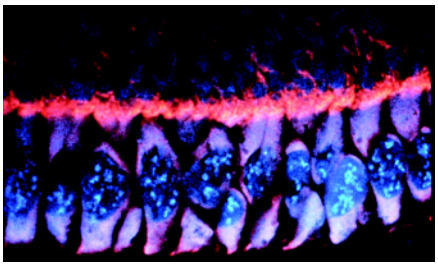


Figure 7.86 The cortical region of the developing follicle of the giant silkmoth *Antheraea polyphemus* stained with a monoclonal antibody against cytoskeletal actin. The primary antibody was visualized using biotin-XX goat anti–mouse IgG antibody (B-2763), followed by incubation with Texas Red streptavidin (S-872). The orange to pink colors in this confocal laser-scanning micrograph show the distribution of cytoskeletal actin in the oocyte cortex and follicle cell cytoplasm. The blue color can be attributed to autofluorescence. Image contributed by Ivo Sauman, Department of Biology, Wesleyan University.

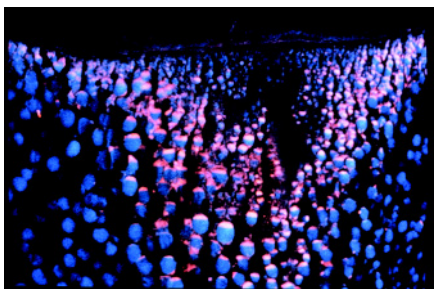


Figure 7.87 The "delta" region of a developing follicle of the giant silkmoth *Hyalophora cecropia* stained with an antibody against the largest subunit of the *Drosophila* RNA polymerase II (RNAp II). The primary antibody was visualized using a biotinylated secondary antibody followed by Texas Red dye–conjugated streptavidin (S-872). The distribution of the RNAp II appears violet because the Texas Red staining colocalizes with the blue autofluorescence of the yolk granules. Image contributed by Ivo Sauman, Biology Department, Wesleyan University.

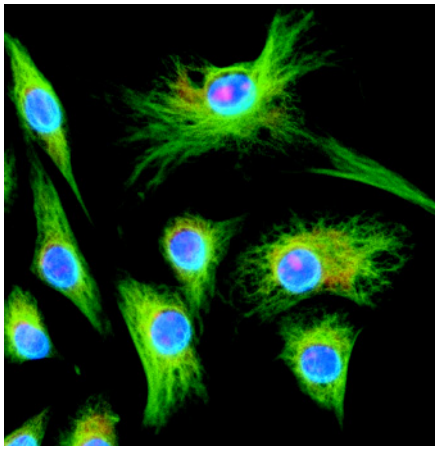


Figure 7.88 Microtubules of fixed bovine pulmonary artery endothelial cells (BPAEC) were localized with mouse monoclonal anti–α-tubulin antibody (A-11126), followed by the biotin-XX–conjugated F(ab′)$_2$ fragment of goat anti–mouse IgG antibody (B-11027) and visualized with the green-fluorescent Alexa Fluor 488 streptavidin (S-11223). The cell was counterstained with blue-fluorescent Hoechst 33342 (H-1399, H-3570, H-21492) to image the DNA and red-fluorescent propidium iodide (P-1304, P-3566, P-21493) to image nucleolar RNA. The multiple-exposure image was acquired using bandpass filter sets appropriate for Texas Red dye, fluorescein and DAPI.

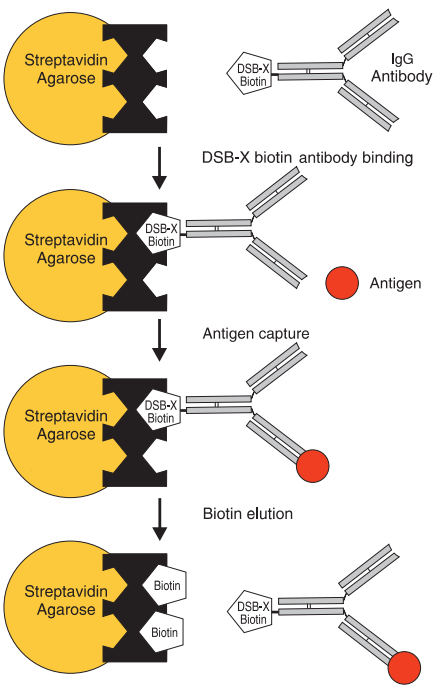


Figure 7.89 Diagram illustrating the use of streptavidin agarose and a DSB-X biotin bioconjugate in affinity chromatography. A DSB-X biotin–labeled IgG antibody and its target antigen are used as an example.

tracer but with much lower affinity for avidin derivatives. The following are commonly used methods for employing avidin, streptavidin, NeutrAvidin biotin-binding protein and CaptAvidin biotin-binding protein as secondary detection reagents:

- **Direct procedure.** A biotinylated or desthiobiotinylated 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, streptavidins or NeutrAvidin biotin-binding proteins or our enzyme-conjugated streptavidins plus a fluorogenic (Figure 7.88), chromogenic or chemiluminescent substrate. Enzyme conjugates of streptavidin are key reagents in some of our Tyramide Signal-Amplification (TSA) Kits (Section 6.2; Table 6.1; Figure 6.11, Figure 6.12, Figure 6.14) and in several of our kits for ultrasensitive detection of proteins on blots (Section 9.4, Table 9.5).
- **Capture and release.** Our unique DSB-X biotin technology (see below) permits the fully reversible labeling of DSB-X biotin derivatives by avidin and streptavidin conjugates (Figure 7.89). Consequently, targets in cells and tissues or on blots labeled with DSB-X biotin conjugates of antibodies (Section 7.3, Table 7.10) or other DSB-X biotin reagents can initially be stained with fluorescent avidin or streptavidin conjugates, then the fluorescent staining can be reversed with D-biotin (B-1595, B-20656; Figure 7.89, Figure 7.90) and the sample restained with an enzyme-conjugated avidin or streptavidin derivative in conjunction with a permanent stain such as diaminobenzidine (DAB, D-22187; Figure 7.91) or the combination of BCIP and NBT (N-6547, Section 10.3).
- **Bridging methods.** A biotinylated antibody or oligonucleotide is used to probe a tissue, cell or other surface. This preparation is then treated with unlabeled avidin,

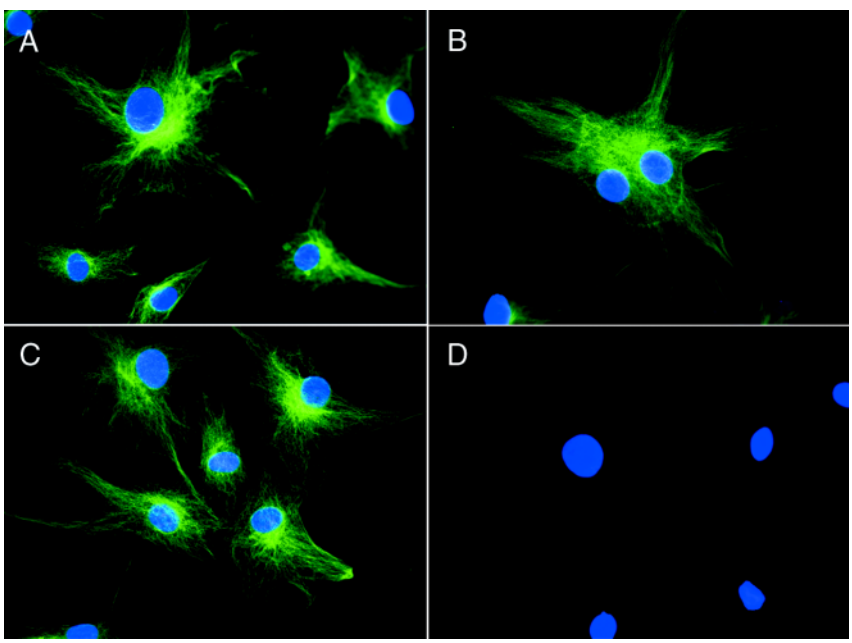


Figure 7.90 Reversible binding by DSB-X biotin. Microtubules of fixed bovine pulmonary artery endothelial cells were labeled with mouse monoclonal anti–α-tubulin antibody (A-11126), detected with either biotin-XX goat anti–mouse IgG antibody (B-2763, panel A) or DSB-X biotin goat anti–mouse IgG antibody (D-20690, panel B) and visualized with green-fluorescent Alexa Fluor 488 streptavidin (S-11223). Nuclei were stained with blue-fluorescent DAPI (D-1306, D-3571, D-21490). After incubating with 10 mM D-biotin (B-1595), the binding between the biotinylated antibody is unaltered (panel C), whereas the streptavidin conjugate has been stripped from the DSB-X biotin–labeled antibody (panel D).

streptavidin or NeutrAvidin biotin-binding protein. Excess reagents are removed by washing, and detection is mediated by a biotinylated detection reagent such as a fluorescent biotin or biocytin dye (Section 4.3), biotinylated R-phycoerythrin (P-811, Section 6.4), biotinylated FluoSpheres microspheres (Section 6.5), or biotinylated horseradish peroxidase (P-917) plus a fluorogenic, chromogenic or chemiluminescent substrate.

- **Indirect procedure.** An unlabeled primary antibody is bound to a cell followed by a biotinylated species-specific secondary antibody. After washing, the complex is detected by one of the two procedures described above. Our Zenon One Biotin-XX and DSB-X Biotin Mouse IgG_1 Labeling Kits (Z-25052, Z-25053; Section 7.2) permit the rapid and quantitative labeling of any mouse IgG_1 antibody for combination with avidin–biotin detection methods.

Endogenous Biotin Blocking Kit

Mammalian cells and tissues contain biotin-dependent carboxylases, which are required for a variety of metabolic functions. These biotin-containing enzymes sometimes produce substantial background signals when avidin–biotin detection systems are used to identify cellular targets [22] (Figure 12.28, Figure 12.30). Because biotin-based technologies can be so sensitive — particularly when using enzyme-amplified detection methods such as TSA — we recommend pre-blocking endogenous biotin present in cells with the reagents in our Endogenous Biotin-Blocking Kit (E-21390). This kit provides streptavidin and biotin solutions in convenient dropper bottles and an easy-to-follow protocol. Sufficient material is provided for approximately one hundred 18 mm × 18 mm glass coverslips.

Fluorescent Conjugates of Biotin-Binding Proteins

Fluorophore-Labeled Avidin, Streptavidin and NeutrAvidin Biotin-Binding Protein

Fluorescent avidin and streptavidin are extensively used in DNA hybridization techniques,[29,30] immunohistochemistry (Figure 7.92) and multicolor flow cytometry.[31–33] Molecular Probes' selection of avidin, streptavidin and NeutrAvidin conjugates keeps growing as we introduce new and improved fluorophores and signal-amplification technology. We continue to provide avidin, streptavidin and NeutrAvidin conjugates of fluorescein, Lissamine Rhodamine B and Texas Red dyes. However, we strongly recommend that researchers evaluate our many newer fluorescent conjugates:

- The green-fluorescent Alexa Fluor 488 (Figure 7.5) and Oregon Green (Figure 7.17) conjugates are not only brighter than fluorescein conjugates, but also much more photostable and less pH sensitive (Section 1.3; see The Alexa Fluor Dye Series — Peak Performance Across the Visible Spectrum in Section 1.3; Figure 7.2, Figure 7.19).
- The Alexa Fluor 430 streptavidin conjugate (S-11237) absorbs maximally at ~434 nm, with bright yellow-green emission (Figure 7.4).
- Other conjugates made with some of our best dyes include those labeled with our orange- to red-fluorescent Alexa Fluor 546 (Figure 7.7), Alexa Fluor 555 (Figure 7.8), Alexa Fluor 568 (Figure 7.9) and Rhodamine Red-X (Figure 7.21) dyes, and with the red-fluorescent Alexa Fluor 594 (Figure 7.10) and Texas Red-X (Figure 7.22) dyes. These conjugates are more fluorescent than traditional Lissamine rhodamine B and Texas Red conjugates (Figure 1.71, Figure 1.78), yet have similar excitation and emission maxima (Figure 1.69).
- Our Alexa Fluor 633 (Figure 7.11), Alexa Fluor 647 (Figure 7.12), Alexa Fluor 660 (Figure 7.13), Alexa Fluor 680 (Figure 7.14), Alexa Fluor 700 (Figure 7.15) and Alexa Fluor 750 (Figure 7.16) conjugates of streptavidin have fluorescence that is not visible to the eye, but their absorption occurs at wavelengths that are easily excited by laser and laser diode light sources (Figure 1.21) and their fluorescence is easily detected by infrared-light–sensitive detectors. Conjugates of the Alexa Fluor 555 and Alexa Fluor 647 dyes, in particular, have fluorescence that is superior to that of the spectrally similar Cy3 and Cy5 dyes (Figure 7.45, Figure 7.46) and are more photostable than Cy3 and Cy5 conjugates.

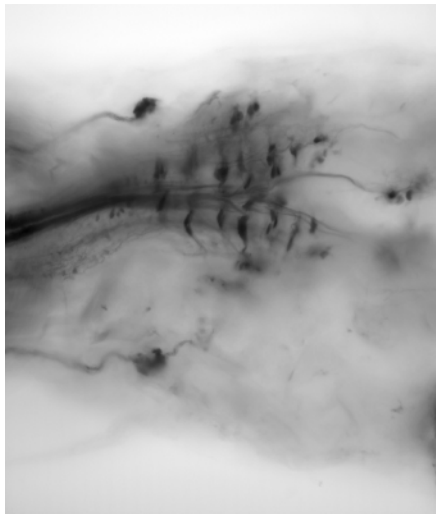


Figure 7.91 Zebrafish embryos were back-filled by incision with horseradish peroxidase (HRP) to trace the spinal neurons. The signal was then developed using diaminobenzidine (DAB) and hydrogen peroxide.

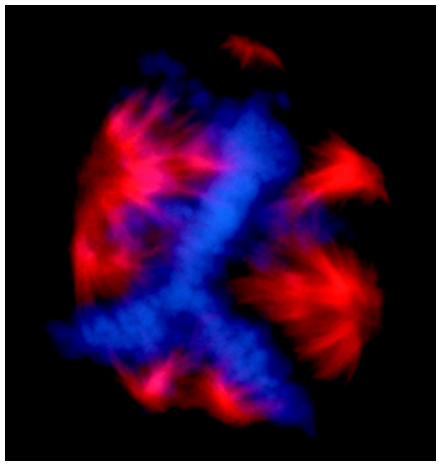


Figure 7.92 A multinucleate HeLa cell in metaphase that was fixed and then stained with a combination of fluorescent dyes. The chromosomes were stained with DAPI (D-1306, D-3571, D-21490). The cytoskeleton was detected with the biotin-XX conjugate of mouse monoclonal anti–α-tubulin antibody (A-21371), which was then visualized with red-fluorescent Alexa Fluor 568 streptavidin (S-11226). The multiple-exposure image was acquired using filter sets appropriate for rhodamine and DAPI.

Molecular Probes prepares streptavidin labeled with 33 different fluorescent dyes and with microspheres and enzymes. See the Product List in this section for a complete list of these products.

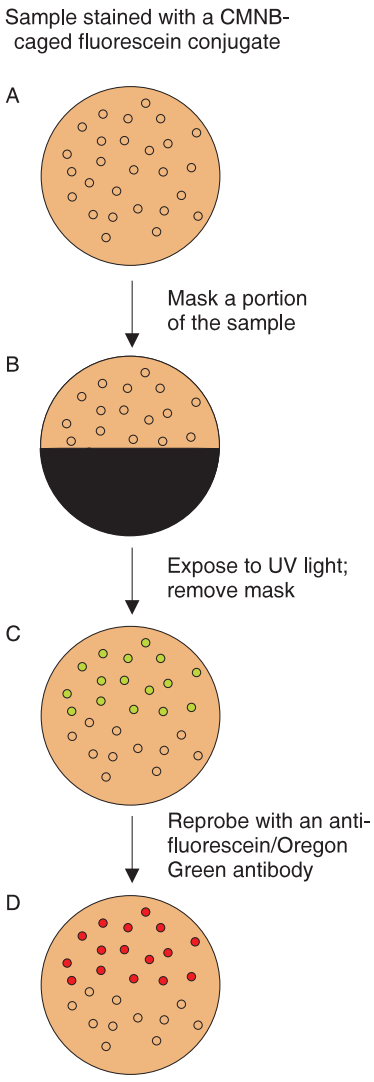


Figure 7.93 Schematic representation of photoactivated fluorescence combined with sample masking. Initially, no fluorescence is observed from samples stained with a CMNB-caged fluorescein-labeled secondary detection reagent (panel A). The desired mask is then placed over the sample (panel B), after which the sample is exposed to UV light. The mask is then removed; fluorescein molecules present in the unmasked portion of the sample are uncaged by the UV light and fluoresce brightly when viewed with the appropriate filters (panel C). Uncaged fluorescein may now also serve as a hapten for further signal amplification using our anti-fluorescein/Oregon Green antibody (A-889). For example, probing with the anti-fluorescein/Oregon Green antibody followed by staining with the Alexa Fluor 594 goat anti–mouse IgG antibody (A-11005) can be used to change the color of the uncaged probe to red fluorescent (panel D).

- For blue-fluorescent labeling, we offer streptavidin and NeutrAvidin conjugates of the Alexa Fluor 350, Marina Blue, Cascade Blue and Pacific Blue fluorophores. In side-by-side testing, our Alexa Fluor 350 streptavidin (S-11249) displays significantly more fluorescence than AMCA streptavidin (Section 1.7, Figure 7.28).
- R-phycoerythrin (R-PE) conjugates (Figure 7.24) of streptavidin (S-866) and NeutrAvidin biotin-binding protein (A-2660) have the most intense fluorescence of all avidin conjugates. They are particularly important labels for multicolor flow cytometry (Section 6.4), MHC tetramer technology (see MHC Tetramer Technology in Section 6.4) and detection of biotinylated nucleic acid probes on nucleic acid microarrays.[34–37] Our "tetramer grade" R-PE conjugate of streptavidin (S-21388) has been purified to ensure that all unconjugated streptavidin has been removed, making it especially suitable for tetramer technology. Additionally, we offer allophycocyanin streptavidin (S-868, Figure 7.27), which can be excited by the 633 nm spectral line of the He–Ne laser.[38] Allophycocyanin conjugates are both brighter and more photostable than Cy5 conjugates, with similar spectra (Figure 6.28).
- We have conjugated R-PE with three of our Alexa Fluor dyes — the Alexa Fluor 610, Alexa Fluor 647 and Alexa Fluor 680 dyes — then conjugated these fluorescent proteins to streptavidin to yield labeled conjugates that can be excited with the 488 nm spectral line of the argon-ion laser (Figure 6.31). The long-wavelength emission maxima are 630 nm for the Alexa Fluor 610–R-PE conjugate (S-20982), 667 nm for the Alexa Fluor 647–R-PE conjugate (S-20992) and 702 nm for the Alexa Fluor 680–R-PE conjugate (S-20985). Emission of the Alexa Fluor 610–R-PE conjugates is shifted to longer wavelengths by about 17 nm relative to that of Texas Red conjugates of R-PE (Figure 6.36). The Alexa Fluor 647–R-PE tandem conjugates have spectra virtually identical to those of Cy5 conjugates of R-PE but are about three times more fluorescent (Figure 6.35). These tandem conjugates can potentially be used for simultaneous four-color labeling with a single excitation (Figure 6.31). In addition, we have reacted allophycocyanin (APC) with our Alexa Fluor 680, Alexa Fluor 700 and Alexa Fluor 750 dyes and then conjugated these labels to streptavidin (S-21002, S-21005, S-21008). The resulting probes can all be excited by the He–Ne laser at 633 nm or krypton-ion laser at 647 nm and have distinguishable emission spectra (Figure 6.34).

A complete list of our current offerings of fluorophore- and enzyme-labeled avidins, streptavidins and NeutrAvidin biotin-binding proteins can be found in Table 7.17. To obtain the maximal fluorescence signal from some conjugates, free D-biotin (B-1595, B-20656) can be added (see Add Free Biotin to Obtain Brighter Signals from Some Fluorescent Avidin Conjugates).

CMNB-Caged Fluorescein Conjugate of Streptavidin

In a photoactivated fluorescence (PAF) experiment, ultraviolet illumination of a non-fluorescent molecule rapidly forms a fluorescent product. The CMNB-caged fluorescein conjugate of streptavidin (S-21380) can be used for the light-mediated tagging of biotinylated single cells or a few cells in tissues or individual targets on microarrays or used as a photoaddressable hapten (Figure 7.93). PAF is measured as an increase in signal, even in the presence of a highly autofluorescent background or other green-fluorescent probes. Furthermore, the fluorescein dye that is liberated serves as a hapten that can be specifically detected and the signal amplified by anti-fluorescein/Oregon Green antibody conjugates (Section 7.4). CMNB-caged fluorescein conjugates of goat anti–mouse IgG antibody and goat anti–rabbit IgG antibody (G-21061, G-21080) are also available (Section 7.3, Table 7.3).

Streptavidin-, NeutrAvidin- and Biotin-Labeled Fluorescent Microspheres

Molecular Probes offers streptavidin, NeutrAvidin and biotin conjugates of the intensely fluorescent FluoSpheres and TransFluoSpheres polystyrene microspheres in a variety of colors and sizes, including our europium and platinum luminescent beads labeled with the NeutrAvidin biotin-binding protein for time-resolved fluorometry (Table 6.8, Table 6.9). Because single fluorescent microspheres can be detected, FluoSpheres and TransFluoSpheres beads have significant potential for ultrasensitive flow cytometry

applications and immunodiagnostic assays.[39,40] They may also be useful as tracers that can be detected with standard enzyme-mediated histochemical methods.

BlockAid blocking solution (B-10710) is a protein-based reagent designed principally for use with our streptavidin-, NeutrAvidin-, biotin- and protein A–labeled FluoSpheres and TransFluoSpheres microspheres. Protein- and other macromolecule-labeled microspheres have hydrophobic regions that may cause them to bind to nontarget surfaces in some experimental systems. Although this nonspecific binding can often be relieved by the use of a blocking solution, we have found that microspheres require a stronger blocking solution than those in common use. In our tests, the BlockAid blocking solution was mixed with streptavidin-labeled FluoSpheres microspheres, which were then used to stain several different cell types for subsequent analysis by flow cytometry. We found the BlockAid blocking solution to be superior to blocking solutions available from other companies, as well as to several standard blocking solutions described in the scientific literature for reducing nonspecific binding of labeled microspheres. BlockAid blocking solution has been found to be effective in flow cytometry applications involving NIH 3T3, A431, RAW and Jurkat cell lines. We expect that the BlockAid blocking solution will be useful for reducing the nonspecific binding of protein-coated or other macromolecule-coated microspheres in a variety of flow cytometry and microscopy applications. It may also be useful as a general blocking agent in a variety of other assays.

NANOGOLD and Alexa Fluor FluoroNanogold Streptavidin

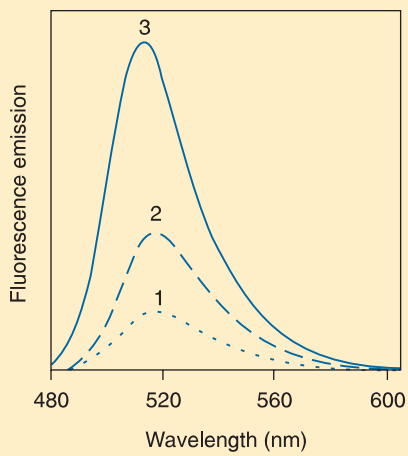
In collaboration with Nanoprobes, Inc., Molecular Probes offers NANOGOLD (N-24918) and Alexa Fluor FluoroNanogold streptavidin (A-24926, A-24927) to help provide clear visibility in immunoblotting, light microscopy, and electron microscopy applications. NANOGOLD gold clusters have several advantages over colloidal gold. They develop better with silver than do most gold colloids and as a result, provide higher sensitivity. Additionally, NANOGOLD particles do not have as high affinity for proteins as do gold colloids, thereby reducing any background due to nonspecific binding. Several additional advantages of NANOGOLD and Alexa Fluor FluoroNanogold streptavidin over colloidal gold conjugates include:

- The NANOGOLD gold clusters are an extremely uniform (1.4 nm ± 10% diameter) and stable compound, not a gold colloid.
- NANOGOLD gold clusters are smaller than an IgG (H+L) antibody and therefore will be able to penetrate cells and reach antigens that are inaccessible to conjugates of larger gold particles.
- NANOGOLD conjugates contain absolutely no aggregates, as they are chromatographically purified through gel filtration columns. This feature is in sharp contrast to colloidal gold conjugates, which are usually prepared by centrifugation to remove the largest aggregates and frequently contain significantly smaller aggregates.
- The ratio of NANOGOLD particle to streptavidin is nearly 1:1, making this product distinct from the 0.2–10 variable stoichiometry of most colloidal gold preparations.
- NANOGOLD cluster–stained targets develop better with silver than do most gold colloids, resulting in higher sensitivity. Silver enhancement, such as the system provided in the LI Silver Enhancement Kit (L-24919, Figure 7.60) can be used for light microscopy and immunoblotting to provide improved results (see Section 7.3).

TECHNICAL NOTE

Add Free Biotin to Obtain Brighter Signals from Some Fluorescent Avidin Conjugates

Fluorophores conjugated to avidin and streptavidin may be quenched significantly, apparently because the dyes interact with amino acid residues in the biotin-binding pocket. Exceptions include Cascade Blue dye– and phycobiliprotein-labeled avidin and streptavidin; the dyes in these conjugates are not quenched because they do not interact with the biotin-binding site. A significant recovery of the avidin or streptavidin conjugate's fluorescence can be obtained if biotin (B-1595, B-20656; Section 4.2) is added as a final incubation step in the staining procedure (see figure). Fluorescence enhancement of avidin conjugates by biotin has been shown to occur in <100 milliseconds.[1] Biotin apparently blocks the interaction of the fluorophore with residues in the biotin-binding pocket that quench the fluorescence, enhancing the fluorescence of the stained tissue, often multifold.



Spectra showing the fluorescence of 1) fluorescein-labeled avidin, 2) fluorescein-labeled avidin after addition of 10 μM biotin and 3) free fluorescein at the same concentration as the fluorescein label in the avidin conjugate.

References

1. Biophys J 69, 716 (1995).

Alexa Fluor FluoroNanogold streptavidin has all the advantages of the NANOGOLD cluster, with the additional benefit that it may be used just like a conventional fluorescent probe. Molecular Probes offers several other NANOGOLD and Alexa Fluor FluoroNanogold reagents (Table 7.9), including the affinity-purified Fab fragments of the goat anti–mouse IgG, goat anti–rabbit IgG and rabbit anti–goat IgG antibodies (Section 7.3) covalently conjugated to the 1.4 nm NANOGOLD gold cluster label. Also available are NANOGOLD mono(sulfosuccinimidyl ester) (N-20130, Section 1.6, Figure 1.84) and NANOGOLD monomaleimide (N-20345, Section 2.2, Figure 2.17), which can be conjugated to amines and thiols, respectively, in the same way that dyes are conjugated to proteins and nucleic acids.

DAB Histochemistry Kits

The use of horseradish peroxidase (HRP) for enzyme-amplified immunodetection, commonly referred to as immunoperoxidase labeling, is a well-established standard histochemical technique.[41,42] The most widely used HRP substrate for these applications is diaminobenzidine (DAB), which generates a brown-colored polymeric oxidation product localized at HRP-labeled sites (Figure 7.61). The DAB reaction product can be visualized directly by bright-field light microscopy or, following osmication, by electron microscopy. We offer DAB Histochemistry Kits for detecting mouse (D-22185) and rabbit (D-22186) IgG primary antibodies and biotinylated antibodies and tracers (D-22187). Each kit contains:

- An HRP-labeled secondary antibody or streptavidin conjugate
- The DAB substrate
- Hydrogen peroxide
- A reaction buffer
- A blocking reagent
- A detailed staining protocol

Each kit provides sufficient materials to stain approximately 200 slides.

Enzyme Conjugates of Biotin-Binding Proteins

Enzyme conjugates are extensively used in enzyme-linked immunosorbent assays (ELISAs),[43] blotting techniques,[44] *in situ* hybridization[45] and cytochemistry and histochemistry.[46] Enzyme-mediated *in situ* techniques using these conjugates provide better resolution and are safer, more sensitive and faster than radioactive methods. Most frequently, the enzymes of choice are horseradish peroxidase, alkaline phosphatase and *Escherichia coli* β-galactosidase because of their high turnover rate, stability, ease of conjugation and relatively low cost. Molecular Probes has prepared highly active enzyme conjugates of streptavidin and NeutrAvidin biotin-binding protein, as well as of biotin-XX. Fluorogenic substrates for ELISAs are often much more sensitive than chromogenic substrates in these important assays. Our fluorogenic, chromogenic and chemiluminescent substrates for these assays are described in Chapter 10.

Our enzyme conjugates of streptavidin and NeutrAvidin biotin-binding protein are prepared by techniques that yield an approximate 1:1 ratio of enzyme to avidin analog, thus ensuring maximum retention of activity of both enzyme and carrier protein. We offer streptavidin conjugates of alkaline phosphatase, horseradish peroxidase and β-galactosidase (S-921, S-911, S-931) and the NeutrAvidin conjugate of peroxidase[47] (A-2664). To decrease background problems, researchers often prefer to use the biotin-XX conjugate of peroxidase (P-917) in conjunction with an avidin or streptavidin bridge for indirect detection of a wide array of biotinylated biomolecules. Our biotinylated peroxidase conjugate is prepared with a reactive biotin-XX derivative, which contains the longest available spacer and allows high avidin affinity.

A principal application of HRP and alkaline phosphatase conjugates of avidins is in enzyme-amplified histochemical staining of cells and tissues. Several of the Tyramide Signal-Amplification (TSA) Kits (Table 6.1) in Section 6.2 and Enzyme-Labeled Fluorescence (ELF) Kits in Section 6.3 utilize enzyme conjugates of streptavidin to yield intensely fluorescent staining of cellular targets (Figure 6.11, Figure 6.12, Figure 6.14, Figure 6.23). These kits are very useful for immunofluorescence, *in situ* hybridization and flow cytometry. Use of a combination of the TSA and ELF technologies or double application of TSA methods promises to provide the highest sensitivity known for detection of low-abundance targets.[48]

Affinity Chromatography

Streptavidin Agarose

Molecular Probes prepares streptavidin conjugated to 4% beaded crosslinked agarose (S-951) — a matrix that can be used to isolate biotinylated peptides, proteins, hybridization probes, haptens and other molecules.[49] In addition, biotinylated antibodies can be bound to streptavidin agarose to generate an affinity matrix for the large-scale isolation of antigens.[49] For instance, staurosporine-treated myotubules have been incubated with biotinylated α-bungarotoxin (B-1196, Section 16.2) in order to isolate the acetylcholine receptors (AChRs) on streptavidin agarose and assess staurosporine's effect on the degree of phosphorylation of this receptor.[50] Streptavidin agarose has also been used to investigate the turnover of cell-surface proteins that had previously been derivatized with an amine-reactive biotin[51] (B-1582, Section 4.2).

DSB-X Bioconjugate Isolation Kit #1

The DSB-X Bioconjugate Isolation Kit #1 (D-20658) uses our unique DSB-X biotin technology for the easy affinity isolation of DSB-X biotin–labeled bioconjugates under extremely gentle conditions. DSB-X biotin is a derivative of desthiobiotin (Figure 4.1), a stable biotin precursor.[52,53] DSB-X biotin utilizes a seven-atom spacer to increase the ability of the DSB-X biotin conjugate to bind in the deep biotin-binding pocket of streptavidin or avidin.[54,55] Whereas harsh chaotropic agents and low pH (6.0 M guanidine HCl, pH 1.5) are required to dissociate a biotin complex from the avidin or streptavidin, streptavidin agarose has only a moderate affinity for conjugates of DSB-X biotin.[56] Therefore, binding can be rapidly reversed by adding excess D-desthiobiotin (D-20657) or natural D-biotin[55] (B-1595, B-20656) to the matrix at neutral pH and at room temperature (or below).

Once bound to the streptavidin agarose matrix, the bioconjugate — an antibody, enzyme, oligonucleotide, nucleic acid, drug or other DSB-X biotin conjugate — can bind to its target, which may be from a variety of sources, including cell or tissue extracts (Figure 7.89). Gentle elution of the entire complex allows subsequent analysis of the affinity-isolated product by electrophoresis or other means. Elution with D-desthiobiotin rather than D-biotin may permit reuse of the matrix.

The DSB-X Biotin Bioconjugate Isolation Kit #1 (D-20658) contains:

- Streptavidin agarose (5 mL of a sedimented bead suspension)
- Solutions of D-desthiobiotin and D-biotin
- Purification columns
- Suggested protocol for binding and release of DSB-X conjugates

Molecular Probes provides a variety of antibody conjugates of DSB-X biotin (Section 7.3, Table 7.10), as well as DSB-X biotin hydrazide (D-20653, Section 3.2), for selective labeling and capture of periodate-oxidized glycoproteins and polysaccharides. Labeling of amine residues of other proteins and other biomolecules is easily accomplished with the reagents in our DSB-X Biotin Protein Labeling Kit (D-20655, Section 1.2).

CaptAvidin Agarose

CaptAvidin agarose (C-21386) is another versatile form of a biotin-binding protein in that its affinity for biotinylated molecules can be completely reversed by raising the pH to 10, permitting the facile separation and isolation of biotin-labeled molecules from complex mixtures (Figure 7.85). This form of agarose-immobilized biotin-binding protein has been used to purify immunoglobulin from whole rabbit serum and to isolate anti-transferrin antibodies directly from rabbit antiserum.[57]

Captivate Ferrofluid Streptavidin

Captivate ferrofluid streptavidin (C-21476) is a versatile product for rapid separation of biotinylated and DSB-X biotin–conjugated biomolecules and their targets from complex mixtures, including those in cell and tissue extracts and bodily fluids. Combination of Captivate ferrofluid streptavidin with DSB-X biotin (Figure 4.1) technology enables the selective capture and release of rare populations of viable cells by DSB-X biotin–conjugated antibodies to cell surface markers (Figure 7.94). A potentially important application of this technique is the detection of specific protein–protein and protein–nucleic acid interactions through selective isolation and release of their intact complexes.

The Captivate ferrofluid products are unique in that they represent the only superparamagnetic particles available that allow both cell sorting and cell-based imaging to be performed simultaneously by use of the Captivate microscope-mounted magnetic yoke assembly and associated Captivate disposable sample chambers (C-24701, C-24700; Section 24.3; Figure 7.62). The Captivate microscope-mounted magnetic yoke assembly includes one free set of 10 disposable sample chambers. Use of Captivate ferrofluid streptavidin in combination with biotin- or DSB-X biotin–conjugated probes permits the simultaneous isolation, visualization and counting of cells that are targets of the antibody by any researcher with access to a standard low-cost microscope with a 10× objective. Also, when used to capture DSB-X biotin–labeled antibodies to cell-surface antigens, the Captivate ferrofluid can be completely separated from the labeled cells by incubation with D-biotin (B-1595, B-20656; Section 4.2) or D-desthiobiotin (D-20657, Section 4.2). The Captivate ferrofluid products should also have advantages over other commercially available magnetic particles in liquid-handling robotic systems.

Molecular Probes has available Captivate magnetic separators [58] for both microplates (C-24702, Figure 24.47) and microtubes (C-24703, Figure 24.48) that we find to be particularly useful with the Captivate ferrofluid products. The microplate separator is compatible with most 96-well microplates, whereas the microtube separator can accommodate six 1.5 mL microcentrifuge tubes. Both separators provide excellent separation efficiency and pull magnetic particles to one side, allowing easier removal of supernatants.[58]

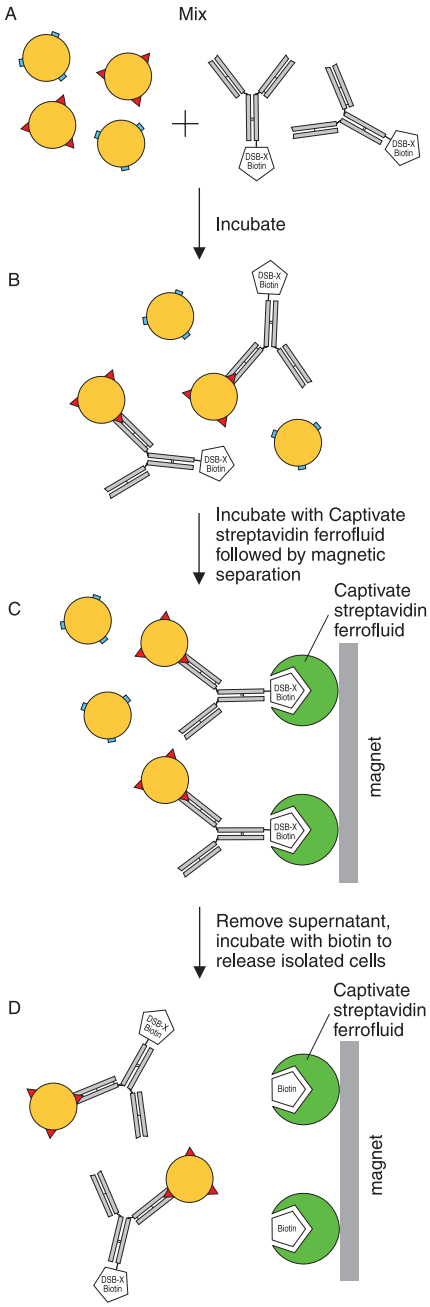


Figure 7.94 Cell separation using Captivate ferrofluid streptavidin and DSB-X biotin conjugates. A mixed population of cells is first mixed with a DSB-X biotin–labeled antibody against an appropriate surface antigen (panel A); subsequent incubation results in the labeling of a specific subpopulation (panel B). The sample is then incubated with a streptavidin-conjugated Captivate ferrofluid (C-21476), which binds to the DSB-X biotin hapten, allowing the labeled cells to be isolated via a magnetic field (panel C). After the unlabeled cells have been washed away, the captured cells can be released by reversing the streptavidin linkage to DSB-X biotin with unlabeled biotin (panel D).

DSB-X Bioconjugate Isolation Kit #2

The DSB-X Biotin Bioconjugate Isolation Kit #2 (D-20659) utilizes DSB-X biotin agarose for affinity isolation of any avidin or streptavidin conjugate (Figure 7.95). DSB-X biotin agarose links desthiobiotin to agarose through a seven-atom spacer (Figure 4.1). Binding of the avidin or streptavidin conjugate can be fully reversed under extremely gentle conditions by addition of D-biotin or desthiobiotin. We have shown that when desthiobiotin (but not D-biotin) is used to reverse the binding the avidin or streptavidin biotin-binding sites are fully saturated by the desthiobiotin; however, it is not necessary to remove the desthiobiotin before use of the affinity-purified avidin or streptavidin conjugate to label a biotin-conjugated probe. The reagents in this kit can be used to isolate streptavidin conjugates that are free from enzymes or other biomolecules when forming protein–protein or protein–nucleic acid conjugates. The DSB-X Biotin Bioconjugate Isolation Kit #2 (D-20659) contains:

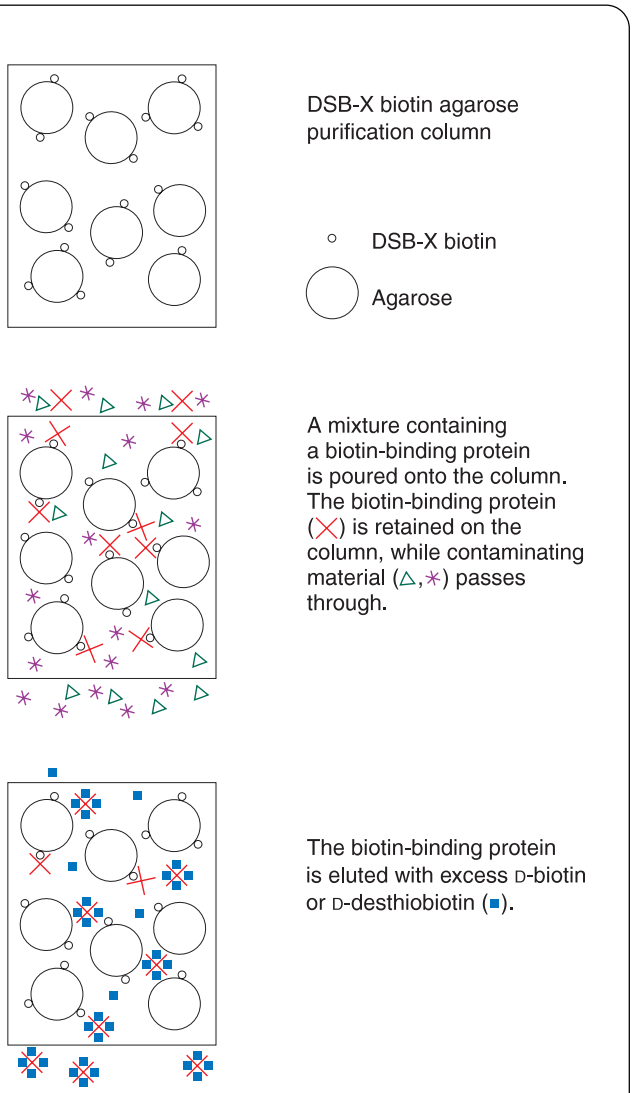


Figure 7.95 Diagram illustrating the use of DSB-X biotin agarose (D-20659) for affinity chromatography.

- DSB-X biotin agarose (5 mL of a sedimented bead suspension)
- Solutions of D-desthiobiotin and D-biotin
- Purification columns
- Suggested protocol for binding and release of DSB-X biotin conjugates

The DSB-X biotin agarose in this kit can potentially be reused several times.

Cell-Surface Biotinylation Kit

Biotin-XX sulfosuccinimidyl ester is a cell-impermeant, amine-reactive compound that can be used to label proteins exposed on the surface of live cells (Figure 4.5). The sulfosuccinidimidyl ester forms an extremely stable conjugate [59] with cell-surface proteins, and the biotin provides a convenient hapten for subsequent isolation or analysis with an avidin-based protein, including streptavidin, NeutrAvidin or CaptAvidin biotin-binding proteins.[60] Cell-surface biotinylation techniques have been employed to differentially label proteins in the apical and basolateral plasma membranes of epithelial cells.[61,62] These techniques are also well suited for studying membrane protein internalization,[60] as well as for investigating the cell-surface targeting of proteins.[63–65]

The FluoReporter Cell-Surface Biotinylation Kit (F-20650) provides a convenient method to label proteins exposed on the cell surface including, but not limited to, membrane proteins. The kit contains biotin-XX sulfosuccinimidyl ester and anhydrous DMSO for preparing stock solutions. The included protocol for cell-surface biotinylation is straightforward and can be completed in less than one hour.

Acrylamide Conjugates for Immobilization of Avidins in Polymers

Streptavidin acrylamide (S-21379), which is prepared from the succinimidyl ester of 6-((*N*-acryloyl)amino)hexanoic acid (acryloyl-X, SE; A-20770, Section 5.2), is a reagent that may be useful for the preparing biosensors.[66] A similar streptavidin acrylamide has been shown to copolymerize with acrylamide on a polymeric surface to create a uniform monolayer of the immobilized protein. The streptavidin can then bind biotinylated ligands, including biotinylated hybridization probes, enzymes, antibodies and drugs. CaptAvidin acrylamide (C-21387) is expected to have similar utility, but offers an advantage — the bond that it forms with biotinylated probes can be reversed at about pH 10.

Biotinylated Reagents for Use with Avidins

In addition to the direct conjugates of avidins, Molecular Probes offers an extensive selection of biotinylated products for use in conjunction with avidins; see Chapter 4 for a complete list of our biotinylation reagents and biotin conjugates. Molecular Probes offers a broad selection of biotinylating reagents, including FluoReporter Biotin-XX and Biotin/DNP Protein Labeling Kits (F-2610, F-6347, F-6348; Section 1.2). Reactive forms of DSB-X biotin and our unique DSB-X biotin bioconjugates are also described in Chapter 4.

ChromaTide Biotin-11-dUTP (C-11411, Section 8.2) can be incorporated into nucleic acids by a variety of enzymatic procedures, providing labeled probes that can be detected by any of our avidin or streptavidin conjugates or whose detection can be amplified using either our TSA (Section 6.2) or ELF technologies (Section 6.3). ChromaTide Biotin-11-dUTP is also incorporated into DNA that has fragmented in the TUNEL assay technique (Section 15.5). The BOLD APB chemiluminescent substrate for membrane-based alkaline phosphatase detection (B-21901, Section 9.4) provides extremely sensitive detection of biotinylated targets when used in combination with our streptavidin conjugate of alkaline phosphatase (S-921).

Our diverse set of biotin and DSB-X biotin conjugates is described in Section 4.3. Combining one of our biotinylated or DSB-X biotin–labeled antibodies (Section 7.3, Table 7.6) with a fluorescent dye– or enzyme-labeled avidin provides an easy method for indirect detection of antibodies from various animal sources. Biotinylated R-phycoerythrin (P-811, Section 6.4) can be used with an avidin or streptavidin bridge to detect biotinylated biomolecules;[67] this bridging technique may substantially reduce the nonspecific staining that is commonly seen when using phycobiliproteins for immunohistochemical applications.

Biotinylated liposomes can be prepared using Molecular Probes' biotin conjugates of phosphoethanolamine (B-1550, B-1616; Section 4.3). Avidin has been used to form a bridge between a biotinylated liposome loaded with fluorescent dyes and a target-specific biotinylated detection reagent. Biotinylated liposomes containing carboxyfluorescein have been employed in an immunoassay that was reported to be both faster and 100-fold more sensitive than the comparable peroxidase-based ELISA.[68]

Anti-Biotin Antibody — An Alternative to Avidins

As an alternative to avidin-based reagents, Molecular Probes offers both unlabeled (A-11242, Section 7.4) and Alexa Fluor 488 dye–labeled (A-11243, Section 7.4) versions of a high-affinity mouse monoclonal antibody to biotin. Our anti-biotin antibody can be used to detect biotinylated molecules in immunohistochemistry, *in situ* hybridization, ELISAs and Western blot applications. It has been shown that certain monoclonal antibodies to biotin have similar biotin-binding motifs as seen for avidin and streptavidin.[69] Anti-biotin antibody has been shown to selectively stain endogenous biotin-dependent carboxylase proteins used in fatty acid synthesis of the mitochondria.[22,70] Nonspecific staining of mitochondrial proteins by labeled avidins and by anti-biotin antibodies can be a complicating factor in using avidin–biotin techniques (Figure 7.84). This nonspecific binding can usually be blocked by pretreatment of the sample with the reagents in our Endogenous Biotin Binding Kit (E-21390; see above). Especially useful for indirect immunofluorescence, the Alexa Fluor 488 conjugate exhibits excitation/emission maxima similar to fluorescein but is brighter, more photostable and its fluorescence intensity is pH insensitive.

Our anti-biotin antibody is a mouse IgG_1–isotype antibody, indicating that its complex with the reagents in any of our Zenon One Mouse IgG_1 Labeling Kits (Section 7.2, Table 7.1) can provide an alternative to avidin- and streptavidin-based reagents for detection of biotinylated probes. Somewhat unexpectedly, our anti-biotin antibody retains high affinity for desthiobiotin and its binding to DSB-X biotin bioconjugates cannot be easily reversed by addition of free D-biotin.

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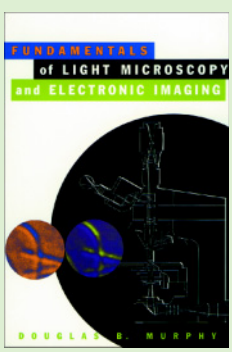
The full citations and, in most cases, links to PubMed for all references in this Handbook *are available at our Web site (www.probes.com/search).*

Cat #	Product Name	Unit Size
A-24926	Alexa Fluor® 488 FluoroNanogold™ streptavidin *80 μg protein/mL*	1 mL
A-24927	Alexa Fluor® 594 FluoroNanogold™ streptavidin *80 μg protein/mL*	1 mL
A-21370	avidin, Alexa Fluor® 488 conjugate	1 mg
A-887	avidin, egg white	100 mg
A-2667	avidin, egg white	5 mg
A-821	avidin, fluorescein conjugate	5 mg
A-11236	avidin, NeutrAvidin™, Alexa Fluor® 350 conjugate	1 mg
A-2666	avidin, NeutrAvidin™ biotin-binding protein	5 mg
A-2663	avidin, NeutrAvidin™, Cascade Blue® conjugate	1 mg
A-2662	avidin, NeutrAvidin™, fluorescein conjugate	1 mg
A-2664	avidin, NeutrAvidin™, horseradish peroxidase conjugate	1 mg
A-11230	avidin, NeutrAvidin™, Marina Blue® conjugate	1 mg
A-6374	avidin, NeutrAvidin™, Oregon Green® 488 conjugate	1 mg
A-6378	avidin, NeutrAvidin™, Rhodamine Red™-X conjugate	1 mg
A-2660	avidin, NeutrAvidin™, R-phycoerythrin conjugate *1 mg/mL*	1 mL
A-6373	avidin, NeutrAvidin™, tetramethylrhodamine conjugate	1 mg
A-2665	avidin, NeutrAvidin™, Texas Red® conjugate	1 mg
A-820	avidin, Texas Red® conjugate	5 mg
B-1595	D-biotin	1 g
B-20656	D-biotin *50 mM aqueous solution*	10 mL
B-10710	BlockAid™ blocking solution *for use with microspheres*	50 mL
C-21387	CaptAvidin™ acrylamide	1 mg
C-21386	CaptAvidin™ agarose *sedimented bead suspension*	5 mL
C-21385	CaptAvidin™ biotin-binding protein	1 mg
C-24701	Captivate™ disposable sample chamber *for Captivate™ magnetic yoke* *set of 10*	1 set
C-21476	Captivate™ ferrofluid streptavidin (streptavidin, Captivate™ ferrofluid conjugate) *0.5 mg Fe/mL*	1 mL
C-24703	Captivate™ magnetic separator for six microtubes	each
C-24702	Captivate™ magnetic separator for 96-well microplates	each
C-24700	Captivate™ microscope-mounted magnetic yoke assembly *includes 10 sample chambers*	each
D-20657	D-desthiobiotin *50 mM aqueous solution*	10 mL
D-22187	Diaminobenzidine (DAB) Histochemistry Kit #3 *with streptavidin–HRP*	1 kit
D-20658	DSB-X™ Bioconjugate Isolation Kit #1 *with streptavidin agarose* *5 isolations*	1 kit
D-20659	DSB-X™ Bioconjugate Isolation Kit #2 *with DSB-X™ biotin agarose* *5 isolations*	1 kit
E-21390	Endogenous Biotin-Blocking Kit *100 assays*	1 kit
F-20650	FluoReporter® Cell-Surface Biotinylation Kit	1 kit
F-8766	FluoSpheres® biotin-labeled microspheres, 0.04 μm, yellow-green fluorescent (505/515) *1% solids*	0.4 mL
F-8767	FluoSpheres® biotin-labeled microspheres, 0.2 μm, yellow-green fluorescent (505/515) *1% solids*	0.4 mL
F-8769	FluoSpheres® biotin-labeled microspheres, 1.0 μm, nonfluorescent *1% solids*	0.4 mL
F-8768	FluoSpheres® biotin-labeled microspheres, 1.0 μm, yellow-green fluorescent (505/515) *1% solids*	0.4 mL
F-20883	FluoSpheres® NeutrAvidin™ labeled microspheres, 0.04 μm, europium luminescent (365/610) *0.5% solids*	0.4 mL
F-20884	FluoSpheres® NeutrAvidin™ labeled microspheres, 0.2 μm, europium luminescent (365/610) *0.5% solids*	0.4 mL
F-20885	FluoSpheres® NeutrAvidin™ labeled microspheres, 1.0 μm, europium luminescent (365/610) *0.5% solids*	0.4 mL
F-20889	FluoSpheres® NeutrAvidin™ labeled microspheres, 0.04 μm, platinum luminescent (390/650) *0.5% solids*	0.4 mL
F-20890	FluoSpheres® NeutrAvidin™ labeled microspheres, 0.2 μm, platinum luminescent (390/650) *0.5% solids*	0.4 mL
F-20891	FluoSpheres® NeutrAvidin™ labeled microspheres, 1.0 μm, platinum luminescent (390/650) *0.5% solids*	0.4 mL
F-8772	FluoSpheres® NeutrAvidin™ labeled microspheres, 0.04 μm, nonfluorescent *1% solids*	0.4 mL
F-8777	FluoSpheres® NeutrAvidin™ labeled microspheres, 1.0 μm, nonfluorescent *1% solids*	0.4 mL
F-8770	FluoSpheres® NeutrAvidin™ labeled microspheres, 0.04 μm, red fluorescent (580/605) *1% solids*	0.4 mL
F-8775	FluoSpheres® NeutrAvidin™ labeled microspheres, 1.0 μm, red fluorescent (580/605) *1% solids*	0.4 mL
F-8771	FluoSpheres® NeutrAvidin™ labeled microspheres, 0.04 μm, yellow-green fluorescent (505/515) *1% solids*	0.4 mL
F-8774	FluoSpheres® NeutrAvidin™ labeled microspheres, 0.2 μm, yellow-green fluorescent (505/515) *1% solids*	0.4 mL
F-8776	FluoSpheres® NeutrAvidin™ labeled microspheres, 1.0 μm, yellow-green fluorescent (505/515) *1% solids*	0.4 mL
F-8780	FluoSpheres® streptavidin-labeled microspheres, 0.04 μm, yellow-green fluorescent (505/515) *0.5% solids*	0.4 mL
L-24919	LI Silver (LIS) Enhancement Kit	1 kit
N-24918	NANOGOLD® streptavidin (streptavidin, NANOGOLD® conjugate) *80 μg protein/mL*	1 mL
P-917	peroxidase from horseradish, biotin-XX conjugate	10 mg
S-888	streptavidin	5 mg
S-21379	streptavidin acrylamide	1 mg
S-951	streptavidin agarose *sedimented bead suspension*	5 mL
S-21002	streptavidin, Alexa Fluor® 680–allophycocyanin conjugate (Alexa Fluor® 680–allophycocyanin streptavidin) *1 mg/mL*	100 μL
S-21005	streptavidin, Alexa Fluor® 700–allophycocyanin conjugate (Alexa Fluor® 700–allophycocyanin streptavidin) *1 mg/mL*	100 μL
S-21008	streptavidin, Alexa Fluor® 750–allophycocyanin conjugate (Alexa Fluor® 750–allophycocyanin streptavidin) *1 mg/mL*	100 μL

Cat #	Product Name	Unit Size
S-11249	streptavidin, Alexa Fluor® 350 conjugate	1 mg
S-11237	streptavidin, Alexa Fluor® 430 conjugate	1 mg
S-11223	streptavidin, Alexa Fluor® 488 conjugate	1 mg
S-11224	streptavidin, Alexa Fluor® 532 conjugate	1 mg
S-11225	streptavidin, Alexa Fluor® 546 conjugate	1 mg
S-21381	streptavidin, Alexa Fluor® 555 conjugate	1 mg
S-11226	streptavidin, Alexa Fluor® 568 conjugate	1 mg
S-11227	streptavidin, Alexa Fluor® 594 conjugate	1 mg
S-21375	streptavidin, Alexa Fluor® 633 conjugate	1 mg
S-21374	streptavidin, Alexa Fluor® 647 conjugate	1 mg
S-21377	streptavidin, Alexa Fluor® 660 conjugate	1 mg
S-21378	streptavidin, Alexa Fluor® 680 conjugate	1 mg
S-21383	streptavidin, Alexa Fluor® 700 conjugate	1 mg
S-21384	streptavidin, Alexa Fluor® 750 conjugate	1 mg
S-20982	streptavidin, Alexa Fluor® 610–R-phycoerythrin conjugate (Alexa Fluor® 610–R-phycoerythrin streptavidin) *1 mg/mL*	100 µL
S-20992	streptavidin, Alexa Fluor® 647–R-phycoerythrin conjugate (Alexa Fluor® 647–R-phycoerythrin streptavidin) *1 mg/mL*	100 µL
S-20985	streptavidin, Alexa Fluor® 680–R-phycoerythrin conjugate (Alexa Fluor® 680–R-phycoerythrin streptavidin) *1 mg/mL*	100 µL
S-921	streptavidin, alkaline phosphatase conjugate *2 mg/mL*	0.5 mL
S-868	streptavidin, allophycocyanin, crosslinked, conjugate *1 mg/mL*	0.5 mL
S-21380	streptavidin, CMNB-caged fluorescein conjugate	1 mg
S-869	streptavidin, fluorescein conjugate	1 mg
S-931	streptavidin, β-galactosidase conjugate	1 mg
S-911	streptavidin, horseradish peroxidase conjugate	1 mg
S-11221	streptavidin, Marina Blue® conjugate	1 mg
S-6368	streptavidin, Oregon Green® 488 conjugate	1 mg
S-6369	streptavidin, Oregon Green® 514 conjugate	1 mg
S-11222	streptavidin, Pacific Blue™ conjugate	1 mg
S-866	streptavidin, R-phycoerythrin conjugate *1 mg/mL*	1 mL
S-871	streptavidin, rhodamine B conjugate	1 mg
S-6366	streptavidin, Rhodamine Red™-X conjugate	1 mg
S-21388	streptavidin, R-phycoerythrin conjugate *tetramer grade* *1 mg/mL*	1 mL
S-870	streptavidin, tetramethylrhodamine conjugate	1 mg
S-872	streptavidin, Texas Red® conjugate	1 mg
S-6370	streptavidin, Texas Red®-X conjugate	1 mg
T-8860	TransFluoSpheres® NeutrAvidin™ labeled microspheres, 0.04 µm (488/605) *1% solids*	0.4 mL
T-8861	TransFluoSpheres® NeutrAvidin™ labeled microspheres, 0.1 µm (488/605) *1% solids*	0.4 mL
T-10711	TransFluoSpheres® streptavidin-labeled microspheres, 0.04 µm (488/645) *0.5% solids*	0.4 mL

Fundamentals of Light Microscopy and Electronic Imaging



Modern research microscopes are advanced electronic imaging systems designed for resolving the structural and functional intricacies of complex biological specimens. Covering optical infrastructure, cameras and image processing techniques from the stand-point of both fundamental principles and practical implementation, this comprehensive 384-page reference provides researchers with the technical background essential for obtaining peak performance from these technically sophisticated instruments. Written in a practical, accessible style, *Fundamentals of Light Microscopy and Electronic Imaging* (F-24840) provides comprehensive coverage of essential topics including:

- Illuminators, filters, and isolation of specific wavelengths
- Phase contrast and differential interference contrast
- Properties of polarized light and polarization microscopy
- Fluorescence and confocal laser-scanning microscopy
- Digital CCD microscopy and image processing

Each chapter includes practical demonstrations and exercises along with a discussion of the relevant material. In addition, a thorough glossary assists with complex terminology and an appendix contains lists of materials, procedures for specimen preparation, and answers to questions.