

7.4 Anti-Dye and Anti-Hapten Antibodies

Anti-Dye and Anti-Hapten Antibodies

In addition to being useful for direct optical detection, some fluorescent and nonfluorescent dyes make excellent haptens that can be recognized by secondary detection reagents in applications such as *in situ* hybridization, enzyme-linked immunosorbent assay (ELISA) techniques and detection of labeled targets on blots (Section 9.4). Antibodies to dyes provide unique opportunities both for signal enhancement and for correlated fluorescence and electron microscopy studies. Essentially all of the methods that use biotin and avidin reagents (Section 7.6) are also possible using dyes as haptens, as long as the corresponding anti-dye antibody is also available (Table 7.13). One advantage of using

fluorescent dyes as haptens instead of biotin-based techniques is that the hapten signal is usually visible, or at least its concentration can be measured by its absorption in solution, preceding the secondary detection step. Unlike biotin, which is an endogenous ligand in mitochondria (Figure 7.84, Figure 12.28), use of dye-based haptens permits background-free staining of cells and tissues. Availability of noncrossreacting antibodies to a variety of haptens is essential for any multicolor application and an alternative to utilizing antibodies against multiple species of animals, which have a higher likelihood of crossreacting. Molecular Probes provides the largest assortment of anti-dye antibodies commercially available (Table 7.13), including rabbit polyclonal IgG antibodies to the fluorescein, tetramethylrhodamine, Texas Red, dansyl, Alexa Fluor 488, BODIPY FL, lucifer yellow and Cascade Blue fluorophores, as well as a goat anti-fluorescein/Oregon Green IgG antibody (A-11095). We also provide antibodies against three nonfluorescent haptens: dinitrophenyl (DNP), biotin and nitrotyrosine (Table 7.14).

Table 7.13 Anti-fluorophore antibodies and their conjugates.

Cat #	Anti-Fluorophore	Host	Label
A-889	Fluorescein/Oregon Green	Rabbit	None
A-11095	Fluorescein/Oregon Green	Goat	None
A-6421	Fluorescein/Oregon Green	Mouse (clone 4-4-20)	None
A-6413	Fluorescein/Oregon Green	Rabbit, Fab fragment	None
A-982	Fluorescein/Oregon Green	Rabbit	Biotin-XX
A-11090	Fluorescein/Oregon Green	Rabbit	Alexa Fluor 488
A-11091	Fluorescein/Oregon Green	Rabbit	Alexa Fluor 594
A-11096	Fluorescein/Oregon Green	Goat	Alexa Fluor 488
A-21250	Fluorescein/Oregon Green	Rabbit	R-phycoerythrin
A-21251	Fluorescein/Oregon Green	Rabbit	Alkaline phosphatase
A-21252	Fluorescein/Oregon Green	Rabbit F(ab') ₂	Alkaline phosphatase
A-21253	Fluorescein/Oregon Green	Rabbit	Horseradish peroxidase
A-21254	Fluorescein/Oregon Green	Rabbit F(ab') ₂	Horseradish peroxidase
A-11094	Alexa Fluor 488	Rabbit	None
A-5770	BODIPY FL	Rabbit	None
A-5750	Lucifer yellow	Rabbit	None
A-5751	Lucifer yellow	Rabbit	Biotin-XX
A-6397	Tetramethylrhodamine and Rhodamine Red	Rabbit	None
A-6399	Texas Red	Rabbit	None
A-6398	Dansyl	Rabbit	None
A-5760	Cascade Blue	Rabbit	None

Antibodies to Fluorescein and Oregon Green Dyes

We have observed complete crossreactivity of our anti-fluorescein antibodies with the Oregon Green 488 and Oregon Green 514 dyes (Section 1.5). The antibodies also quench the fluorescence of the structurally similar dye, resorufin (R-363, Section 10.1, Figure 10.5). The high affinity and specificity of anti-fluorescein/Oregon Green antibodies (A-889, A-6413, A-6421, A-11095) makes fluorescein and Oregon Green dyes ideal haptens for various detection schemes.^{1,2} Researchers have found that fluorescein-anti-fluorescein ELISA techniques display low non-specific binding and are similar in sensitivity to biotin-streptavidin methods.³ In addition to our anti-fluorescein/Oregon Green rabbit polyclonal antibody (A-889) and anti-fluorescein/Oregon Green goat polyclonal antibody (A-11095), Molecular Probes offers an anti-fluorescein/Oregon green monoclonal antibody and a rabbit polyclonal anti-fluorescein/Oregon Green Fab fragment (see Antibody Structure and Classification in Section 7.3). The high-affinity anti-fluorescein/Oregon Green mouse monoclonal 4-4-20 antibody (A-6421) may reduce nonspecific binding in ELISAs and other second-step detection assays. The Fab fragment of our polyclonal anti-fluorescein/Oregon green antibody (A-6413) provides researchers with a probe that more efficiently penetrates cell and tissue preparations. Furthermore, because the

Table 7.14 Molecular Probes' anti-hapten antibodies and conjugates.

Cat #	Anti-Hapten	Host	Label
A-11242	Biotin	Mouse	None
A-11243	Biotin	Mouse	Alexa Fluor 488
A-6430	Dinitrophenyl	Rabbit	None
A-6435	Dinitrophenyl	Rabbit	Biotin-XX
A-6423	Dinitrophenyl	Rabbit	Fluorescein
A-11097	Dinitrophenyl	Rabbit	Alexa Fluor 488
A-21285	Nitrotyrosine	Rabbit	None

Fab fragment no longer contains the Fc portion, nonspecific interactions with Fc receptor-bearing cells are eliminated. As expected, none of our anti-fluorescein/Oregon Green antibodies recognize the Alexa Fluor or BODIPY dyes.

We also offer the horseradish peroxidase (HRP) and alkaline phosphatase conjugates of the rabbit anti-fluorescein/Oregon Green antibody as both the full IgG (H+L) antibody conjugates (A-21251, A-21253) and as conjugates of the F(ab')₂ antibody fragment (A-21252, A-21254). Enzyme conjugates are commonly used in histochemical amplification schemes such as the tyramide signal-amplification (TSA) technology (Section 6.2) for HRP and the enzyme-labeled fluorescence (ELF) technology (Section 6.3) for alkaline phosphatase. The TSA and ELF technologies provide a greater degree of resolution than many conventional enzyme-mediated fluorescence staining methods, and sequential TSA followed by ELF or by a second round of TSA provides the most sensitive assays available for detection of low-abundance targets in cells and tissues with high spatial resolution⁴ (Figure 6.6). Additionally, these anti-dye antibody conjugates of enzymes can be utilized in ELISAs. Enzyme-conjugated detection reagents often provide greater sensitivity than that achieved with direct dye conjugates. The substrates used to detect the enzymatic activity — and indirectly the amount of the target — typically yield a soluble fluorophore or chromophore. Molecular Probes' assortment of substrates for use in ELISAs is described in Chapter 10.

Our Alexa Fluor 488 dye-labeled rabbit or goat anti-fluorescein/Oregon Green antibodies (A-11090, A-11096) can be used to enhance the green-fluorescent signal of the fluorescein hapten without changing its fluorescence color⁵⁻⁷ (Figure 7.52). Thus, this conjugate allows researchers to take advantage of the superior photostability of the Alexa Fluor 488 dye, while utilizing existing fluorescein-labeled probes and fluorescein-compatible optics. This strategy has been exploited in our Alexa Fluor 488 Signal-Amplification Kit for Fluorescein-Conjugated Probes (A-11053, Section 7.3 and Figure 7.52). Alexa Fluor 488 dye-labeled anti-fluorescein/Oregon Green antibodies (A-11096) can be used with fluoresceinated probes to greatly enhance the photostability of the green-fluorescent signal. The Alexa Fluor 594 dye-labeled (A-11091) anti-fluorescein/Oregon Green antibody can be used to convert the green fluorescence of fluorescein conjugates into photostable red fluorescence, or potentially to amplify the signal from fluorescein conjugates (Figure 7.63).

The R-phycoerythrin conjugate of the rabbit anti-fluorescein/Oregon Green IgG antibody (A-21250) also has the unique utility of both shifting the green-fluorescence emission of fluorescein-labeled probes to longer wavelengths and greatly intensifying the long-wavelength signal (Figure 7.64). Biotin-XX-labeled rabbit anti-fluorescein/Oregon Green antibody (A-982) is an excellent reagent for converting a fluorescence-based detection method into an enzyme-amplified light or electron microscopy technique. Biotin-XX anti-fluorescein/Oregon Green can be combined with either the tyramide signal-amplification (TSA) technology (Section 6.2, Figure 6.6) or enzyme-labeled fluorescence (ELF) technology (Section 6.3, Figure 6.21) in a variety of signal-amplification schemes for cell and tissue labeling.

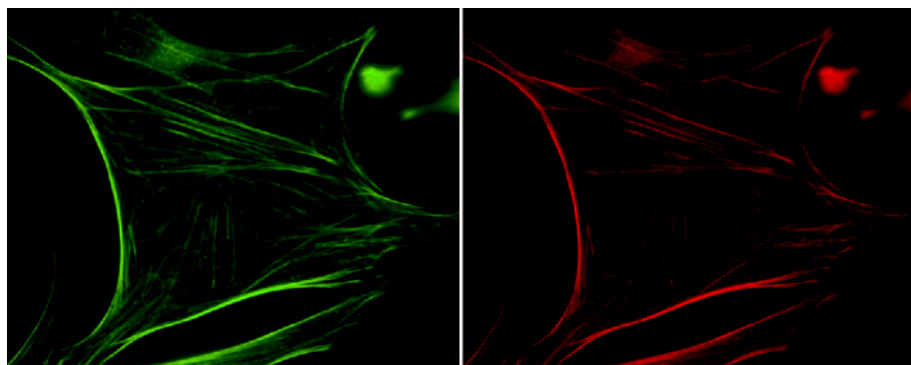


Figure 7.63 Fixed and permeabilized bovine pulmonary arterial endothelial cells were labeled with the filamentous actin (F-actin) stain, fluorescein phalloidin (F-432, left). An Alexa Fluor 594 anti-fluorescein/Oregon Green rabbit IgG antibody (A-11091) converted the green fluorescence to red (right).

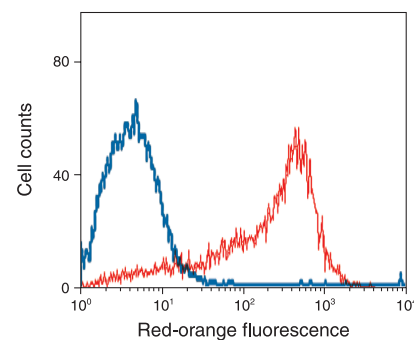
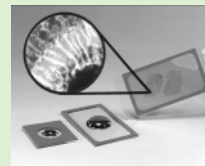


Figure 7.64 Color-shifting using a labeled anti-fluorescein/Oregon Green antibody. Jurkat cells were first stained with a primary mouse anti-human CD3 antibody, followed by fluorescein goat anti-mouse IgG antibody (F-2761), with the resultant fluorescence detected in the R-phycoerythrin (red-orange fluorescence) channel of a flow cytometer (blue curve). The weak signal was then shifted to better suit the R-phycoerythrin channel by the addition of an R-phycoerythrin conjugate of anti-fluorescein/Oregon Green antibody (A-21250). The resulting signal intensity is approximately two orders of magnitude greater (red curve) than the direct fluorescence from the first staining step (blue curve).

CoverWell Incubation Chamber Gaskets



CoverWell incubation chamber gaskets are silicone gaskets with a clear plastic cover that are expressly designed for immunocytochemistry and in situ hybridization. The gasket is simply pressed onto a wet or dry microscope slide to form a watertight chamber that holds reactants in place and prevents evaporation. The chambers improve the uniformity and sensitivity of staining by enclosing a large sample area while minimizing the reagent volume required. The incubation chamber gaskets are easily removed and reapplied for multiple-step procedures. These chamber gaskets are heat resistant, autoclavable and nuclease free. CoverWell incubation chamber gaskets are available with circular (13 mm diameter, 0.2 mm deep, C-18155; 13 mm diameter, 0.5 mm deep, C-18156) or rectangular (40 mm × 22 mm, 0.2 mm deep, C-18150; 40 mm × 22 mm, 0.5 mm deep, C-18151) recesses.

Some of the more important applications for anti-fluorescein/Oregon Green antibodies — almost all of which could also be carried out with any of our other anti-dye antibodies and their complementary dyes — include:

- Amplification of the signal from a fluorescein tyramide in the TSA technology⁸ (Section 6.2)
- Detection of fluorescein-labeled primary antibodies^{9,10}
- Development of fluorescein-labeled cell preparations for electron microscopy¹¹
- Investigation of the uptake of a fluorescein dextran in kidney proximal tubules¹²
- Localization of mRNA sequences in a double *in situ* hybridization experiment in which both fluorescein- and biotin-labeled oligonucleotides were used¹
- Preparation of an anti-fluorescein affinity matrix, which was used to immobilize a fluoresceinated protein in order to study its protein–protein interactions *in vitro*^{13,14}
- Separation of fluorescein antibody–labeled cell populations by immunoadsorption¹⁵
- Assessment of the accessibility of active-site-bound fluorescein probes¹⁶
- Investigation of the internalization pathway of fluorescein transferrin^{17,18} (T-2871, Section 16.1)

Ultraviolet photolysis of the nonfluorescent CMNB-caged fluorescein conjugates of goat anti–mouse IgG antibody and goat anti–rabbit IgG antibody (G-21061, G-21080; Section 7.3) or streptavidin (S-21380, Section 7.6) results in formation of green-fluorescent fluorescein conjugates. 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. This unique photoactivation procedure permits the light-mediated generation of a hapten at selected sites, a process similar in utility to photolithography (Figure 7.93). The DMNB-caged fluorescein dextran conjugates (D-3310, D-7146; Section 14.5) may have similar utility as light-generated haptens. Caged-fluorescein conjugates of other biomolecules can be prepared using the succinimidyl ester of CMNB-caged fluorescein (C-20050, Section 1.4, Figure 1.55).

The anti-fluorescein/Oregon Green antibodies can potentially be used to significantly amplify the signal from nucleic acids labeled by our Oregon Green fluorophores or of fluorescein-labeled nucleic acid probes. These probes include nucleic acids prepared using the following reagents and kits, all of which are discussed in Section 8.2:

- ChromaTide Oregon Green 488-5-dUTP (C-7630)
- ARES Oregon Green 488 DNA Labeling Kit (A-21674)
- ULYSIS Oregon Green 488 Nucleic Acid Labeling Kit (U-21659)

Antibodies to Tetramethylrhodamine and the Rhodamine Red and Texas Red Dyes

As with the anti-fluorescein/Oregon Green antibodies, the rabbit polyclonal antibodies to the tetramethylrhodamine and Texas Red fluorophores (A-6397, A-6399) are effective reagents for binding these dye-based haptens and quenching their fluorescence. However, these antibodies strongly crossreact with some other rhodamines, including the Rhodamine Red and Lissamine

rhodamine B dyes, and therefore cannot be used for simultaneous detection of more than one rhodamine-based dye. These anti-tetramethylrhodamine and anti–Texas Red antibodies do not appear to crossreact with fluorescein or the Oregon Green or Alexa Fluor dyes, and our anti-fluorescein/Oregon Green antibodies do not crossreact with tetramethylrhodamine or the Rhodamine Red or Texas Red dyes. Anti-tetramethylrhodamine has been used to localize retrogradely transported tetramethylrhodamine dextrans by an immunoperoxidase-based amplification technique.¹⁹ These antibodies should also complex with FISH probes labeled with the TAMRA, ROX, Texas Red and Spectrum Orange dyes (Section 8.2, Section 8.5) but not with probes prepared from the sulfonated Alexa Fluor dyes.

Antibody to the Alexa Fluor 488 Dye

Molecular Probes has prepared a rabbit polyclonal antibody to our green-fluorescent Alexa Fluor 488 dye (A-11094). In a manner analogous to the anti-fluorescein/Oregon Green antibodies, the anti–Alexa Fluor 488 antibody specifically recognizes and efficiently quenches most of the fluorescence of the Alexa Fluor 488 dye. In contrast, the anti–Alexa Fluor 488 antibody does not appreciably quench the fluorescence of fluorescein, carboxytetramethylrhodamine (TAMRA) or the Alexa Fluor 594 dye. The high affinity of the anti–Alexa Fluor 488 antibody makes it potentially useful for various immunochemical applications. This antibody can be used to further amplify the signals from our Alexa Fluor 488 tyramide-containing TSA Kits (Section 6.2, Table 6.1), as well as that of labeled nucleic acids prepared from our ARES Alexa Fluor 488 Kit (A-21665, Section 8.2), ULYSIS Alexa Fluor 488 Kit (U-21650, Section 8.2), Alexa Fluor 488 Oligonucleotide Amine Labeling Kit (A-20191, Section 8.2) or our ChromaTide Alexa Fluor 488 UTP, ChromaTide Alexa Fluor 488 OBEO-dCTP and ChromaTide Alexa Fluor 488 dUTP nucleotides (C-11403, C-21555, C-11397; Section 8.2).

Antibodies to the Lucifer Yellow and Cascade Blue Dyes

Lucifer yellow CH (L-453) and Cascade Blue hydrazide (C-687) are frequently employed as polar tracers for neuronal cell labeling (Section 14.3; see Anti–Lucifer Yellow, Anti–Cascade Blue and Anti–Alexa Fluor 488 Antibodies in Section 14.3). Our unconjugated (A-5750, A-5760) and biotinylated (A-5751) rabbit polyclonal antibodies to these dyes are useful in standard enzyme-mediated immunohistochemical methods for permanently labeling neuronal tissue.^{20–24} Anti–lucifer yellow antibody (A-5750) has also been used to follow dye coupling in smooth muscle cells by electron microscopy.^{25,26} The anti–Cascade Blue antibody (A-5760) has been employed in Western blot analysis (Section 9.4) to identify cytoplasmic and luminal domains of the sarcoplasmic reticulum Ca²⁺-ATPase, which had been photolabeled with Cascade Blue aminoethyl 4-azidobenzamide.²⁷ Our Vybrant Cell Lineage Tracing Kit (V-22915, Section 14.5) utilizes a Cascade Blue dextran conjugate and the anti–Cascade Blue antibody for cell-lineage-tracing studies (Figure 7.65). Fluorescence of nucleic acid probes prepared from ChromaTide Cascade Blue dUTP (C-7612, Section 8.2) can be amplified using our anti–Cascade Blue antibody.

Antibody to the BODIPY FL Dye

Our unlabeled rabbit polyclonal antibody to the BODIPY FL fluorophore (A-5770) crossreacts with some other BODIPY dyes

but does not crossreact appreciably with any other fluorophores. This anti-BODIPY FL antibody should therefore not be used for simultaneous detection of more than one dye based on the BODIPY fluorophore. In solution-based assays, we have found that the anti-BODIPY FL antibody effectively quenches most of the fluorescence of the BODIPY FL dye, but quenches the BODIPY TR dye to a lesser degree and does not significantly quench the BODIPY TMR dye. The anti-BODIPY FL antibody has been used in a fluorescence quenching assay to determine the accessibility of BODIPY FL dye-labeled cysteine residues in the transmembrane domain of diphtheria toxin.^{28–31} This antibody should be particularly useful for detection schemes that amplify the signals from nucleic acids that have incorporated the ChromaTide BODIPY FL-14-UTP and ChromaTide BODIPY FL-14-dUTP nucleotides (C-7613, C-7614; Section 8.2). Other applications of the anti-BODIPY FL antibody should include many of those in which the BODIPY FL dyes are used as a simple hapten, such as those described above for anti-fluorescein/Oregon Green antibodies.

Anti-Dansyl Antibody

In contrast to the other anti-fluorophore antibodies, which usually quench the fluorescence of the dye to which they bind, our rabbit polyclonal anti-dansyl antibody (A-6398) typically *enhances* the fluorescence of dansyl amides by greater than 10-fold. Binding of the anti-dansyl antibody also blue shifts the emission spectrum of the fluorophore in water from ~520 nm to ~450 nm. These properties, combined with the unusually high Stokes shift of the dansyl dye (Figure 7.66), make this antibody particularly useful for determining the topography of dansyl-labeled probes, including that of dansyl-labeled phospholipids (Section 13.2) in cell and artificial membranes.³² The dansyl hapten is preferably incorporated into biomolecules using the succinimidyl ester of dansyl-X (D-6104, Section 4.2) because its aminohexanoyl spacer (“X”) reduces the interaction of the fluorophore with the biomolecule to which it is conjugated and makes it more accessible to anti-dansyl antibodies.^{32–34}

Efficient Quenching by Anti-Fluorophore Antibodies

Quenching Efficiencies

Except for the anti-dansyl antibody, which enhances the fluorescence of the dansyl fluorophore, all of our anti-fluorophore antibodies strongly quench the fluorescence of their complementary dyes in free solution. For example, our anti-fluorescein/Oregon Green antibodies typically effect up to 95% quenching of the fluorescence of both fluorescein and the Oregon Green 488 dye. The anti-fluorescein/Oregon Green antibody also quenches some other fluorescein derivatives, such as carboxyfluorescein, Calcium Green-1 and BCECF, making this antibody useful for reducing background fluorescence caused by leakage of these dyes from the cell.³⁵ However, quenching of our fluorescein-based Ca²⁺ indicators by our anti-fluorescein/Oregon Green IgG antibody is apparently dependent on whether or not Ca²⁺ is bound; Calcium Green-1 is quenched by 89% in the presence of 5 μM Ca²⁺, whereas it is quenched by only 46% in the presence of 10 mM EGTA. Maximal quenching efficiencies for fluorescein analogs (all at 5 nM dye using the rabbit anti-fluorescein/Oregon Green IgG antibody, A-889) are as follows (values may vary somewhat from batch to batch) and may be different using the goat anti-fluorescein/Oregon Green IgG antibody, A-11095, the mouse monoclonal anti-fluorescein/Oregon Green, A-6421 or the rabbit IgG Fab fragment of anti-fluorescein/Oregon Green, A-6413):

- Oregon Green 488 dye, 95%
- Oregon Green 514 dye, 92%
- Carboxyfluorescein, 93%
- Calcium Green-1 (in the presence of 5 μM Ca²⁺), 89%
- Calcium Green-1 (in the presence of 10 mM EGTA), 46%
- BCECF, 43%
- Fluo-3 (in the presence of 5 μM Ca²⁺), 32%
- Rhodamine Green dye, 9%
- Calcein, <5%
- Tetramethylrhodamine, <5%

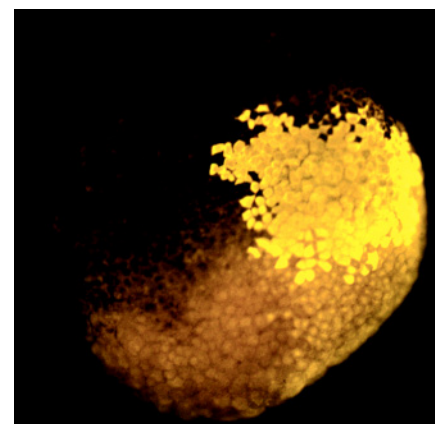


Figure 7.65 Lineage tracing in an African clawed frog (*Xenopus laevis*) embryo performed using the Vybrant Cell Lineage Tracing Kit (V-22915). Embryos at the 8-cell stage were injected with anionic, lysine-fixable Cascade Blue 10,000 MW dextran in one dorsal-animal blastomere and allowed to develop to various stages before being fixed. The Cascade Blue dye, which serves as an antigen in this technique, was detected with an antibody to the Cascade Blue dye and subsequently visualized with a secondary antibody conjugated to the Alexa Fluor 546 dye (A-11010). This photographic image was taken using a bandpass filter set appropriate for rhodamine. Image contributed by Paul Wilson, Cornell University Medical College, New York, and Greg Cox, Molecular Probes, Inc.

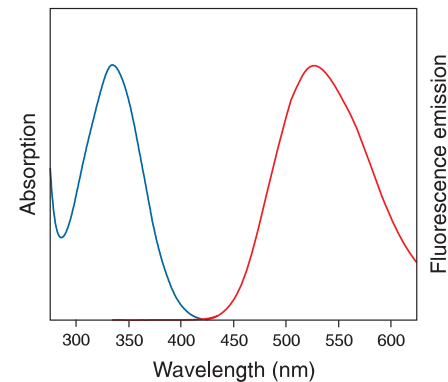


Figure 7.66 Absorption and fluorescence emission spectra of dansyl cadaverine (D-113) in methanol.

Dyes can be used in many of the same detection schemes as with avidin-biotin methods with the added advantages that the hapten can often be detected prior to signal amplification and, unlike biotin, there is no endogenous ligand in cells.

Our preparations of the anti-tetramethylrhodamine and anti-Texas Red antibodies are somewhat less effective as fluorescence quenchers of their complementary fluorophores, with maximal quenching efficiencies of ~75% and ~60%, respectively. Our rabbit anti-Alexa Fluor 488 IgG antibody quenches the fluorescence of the free Alexa Fluor 488 dye by >90%. Our antibody to the BODIPY FL fluorophore typically quenches the dye's fluorescence by ~90%. It also quenches BODIPY TR dye fluorescence by ~45%, but does not significantly quench BODIPY TMR dye fluorescence. Antibodies to the lucifer yellow and Cascade Blue fluorophores quench the fluorescence of their complementary dyes by ~85% and ~80%, respectively. In addition, anti-DNP antibodies have been reported to significantly quench the fluorescence of aminonitrobenzoxadiazoles (NBD amines).³⁶

Quenching Assay

Molecular Probes uses a sensitive fluorescence quenching based-assay to ensure that the concentration of specific anti-dye antibody in its purified IgG fractions is provided at a consistently high titer value. As supplied, 20 μL of the antibody solution is certified to produce $\geq 50\%$ of the maximal fluorescence quenching (or enhancement, in the case of anti-dansyl antibody) of 1 mL of a 50 nM solution of the corresponding dye, assayed in 100 mM sodium phosphate, pH 8.0. All maximal quenching values are determined using the free fluorophore; the maximal quenching of a fluorophore covalently bound to a protein is often significantly less due to steric hindrance.

This fluorescence-quenching assay cannot be applied to our fluorophore-labeled anti-fluorescein/Oregon Green IgG antibody conjugates or to our anti-fluorescein/Oregon Green monoclonal antibody (A-6421); these products typically contain 0.5 mg of total protein.

Applications for Fluorescence Quenching by Anti-Fluorophore Antibodies

Fluorescence quenching of dye haptens by anti-dye antibodies provides a useful measure of topography in cells, proteins and membranes. For example, researchers have used anti-fluorescein quenching assays to determine the accessibility of a fluorescein-labeled ATP-binding site in both Na^+/K^+ -ATPase and $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase.^{16,37} Similarly, the anti-BODIPY FL dye antibody has been employed to identify shallow and deep membrane-penetrating forms of diphtheria toxin T domain.²⁸⁻³¹ In addition, anti-fluorophore antibodies have been used as cell-impermeant probes for determining whether fluorescent dye-conjugated ligands, proteins, bacteria or other biomolecules have been internalized by endocytic or pinocytic processes³⁸⁻⁴¹ (Section 16.1). Anti-fluorophore antibodies also permit background-free observation of fusion events in an assay designed to monitor the fusion of membrane vesicles *in vitro*.⁴² However, as noted above, these antibodies may quench dye-labeled proteins less effectively than they quench free dyes.

Anti-Dinitrophenyl Antibody

Because of its high affinity for the dinitrophenyl (DNP) hapten,^{43,44} our anti-DNP polyclonal rabbit antibodies (Table 7.14) are excellent reagents for probing DNP-labeled molecules, including nucleic acid probes prepared using our ChromaTide dinitrophenyl-11-dUTP nucleotide (C-7610, Section 8.2). Unlike assays that use biotin as the hapten, it is usually easy to determine the degree of substitution of the DNP hapten in bioconjugates from the dye's visible absorption near 350 nm⁴⁵ ($\epsilon \sim 18,000 \text{ cm}^{-1}\text{M}^{-1}$). It has been reported that human chromosomes can be probed with equal sensitivities using either biotinylated, DNP-modified or digoxigenin-modified cosmid probes.⁴⁶ Anti-DNP antibodies have been used to localize a DNP-labeled DNA probe in HIV-infected cells⁴⁷ and 2,4-dinitrophenylhydrazine-labeled proteins on blots.^{48,49} Researchers have also reported using anti-DNP antibodies to probe for DNP-labeled IgG as a method for detecting sparse antigens⁵⁰ and, in conjunction with DNP-labeled bovine serum albumin (BSA), to study the Fc receptor-mediated endocytosis of IgG complexes.^{51,52}

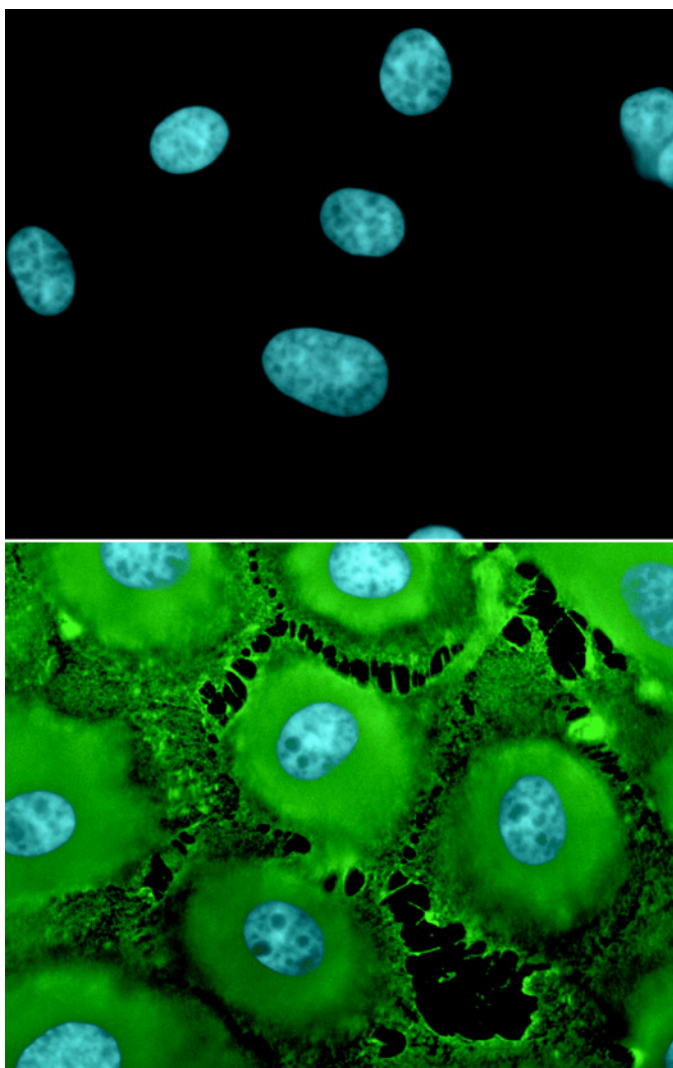


Figure 7.67 Fixed and permeabilized bovine pulmonary artery endothelial cells were treated with either degraded peroxy-nitrite (top panel) or ~100 μM peroxy-nitrite (bottom panel) for five minutes at room temperature to induce protein nitration. Nitrated tyrosine residues were detected with our rabbit anti-nitrotyrosine antibody (A-21285) and visualized with the green-fluorescent Alexa Fluor 488 goat anti-rabbit IgG antibody (A-11008). Nuclei were counterstained with blue-fluorescent DAPI (D-1306, D-3571, D-21490).

A major application of our anti-DNP antibody and its conjugates is expected to be detection of the DNP hapten that is generated by HRP-mediated deposition of DNP-X tyramide, the key reagent in our TSA Kits #34, #35 and #36 (T-20944, T-20945, T-20946; Section 6.2). The DNP-X tyramide-containing TSA Kits have the significant advantage over biotin-XX tyramide-containing TSA Kits in that there is no endogenous DNP in cells, as is the case when biotin is the hapten (Figure 7.84, Figure 12.28).

In addition to the unlabeled anti-DNP antibody (A-6430), Molecular Probes offers anti-DNP antibody conjugates of biotin-XX (A-6435), fluorescein (A-6423) and the Alexa Fluor 488 dye (A-11097). Our anti-DNP antibody is prepared against DNP-keyhole limpet hemocyanin (DNP-KLH) and thus the antibody and its conjugates do not crossreact with BSA, a common blocking reagent in hybridization applications.

For use in conjunction with our anti-DNP antibody, Molecular Probes offers the DNP-X succinimidyl ester (D-2248, Section 4.2) for labeling proteins and amine-modified DNA. The literature also describes methods for incorporating DNP into DNA using DNP-labeled primers.⁵³ In addition to recognizing DNP, our anti-DNP antibody crossreacts with trinitrobenzenesulfonic acid-modified proteins, making this antibody useful both for localizing and for isolating cell-surface molecules labeled with either DNP or TNP.⁵³ Furthermore, anti-DNP antibodies have been reported to quench aminonitrobenzoxadiazoles (NBD amines),³⁶ indicating that NBD-X succinimidyl ester (S-1167, Section 1.7) will also be a useful haptensylating reagent for use with this antibody.

Anti-Nitrotyrosine Antibody

Our antibody to nitrotyrosine (A-21285) is raised in rabbits that have been immunized with nitrated KLH. Nitrotyrosine-modified proteins are the principal reaction products of nitric oxide (through the formation of peroxynitrite) in cells (Section 19.3). Because tyrosine residues are conveniently converted to nitrotyrosine by reaction at near-neutral pH with tetranitromethane,^{54,55} the nitrotyrosine hapten can be readily created in almost any peptide or proteins that contains a tyrosine residue. A further advantage is that nitrotyrosine has pH-dependent visible absorbance (absorption maxima ~360 nm⁵⁴ and 428 nm⁵⁶) that can be utilized to detect formation of the hapten in soluble biopolymers. Our anti-nitrotyrosine antibody is useful for detection of nitrotyrosine-containing proteins both in cells (Figure 7.67) and on Western blots (Section 9.4, Figure 19.20).

Anti-Biotin Antibody

The high affinity of avidin for biotin was first exploited in histochemical applications in the early 1970's.^{57,58} The use of avidin-biotin techniques has since become standard for diverse detection schemes, although limitations of this method have also been recognized.^{59,60} As an alternative to avidin reagents, Molecular Probes offers both unlabeled (A-11242) and Alexa Fluor 488 dye-labeled (A-11243) versions of the high-affinity mouse monoclonal 2F5 antibody to biotin. Monoclonal 2F5 can potentially be used to detect biotinylated molecules in immunohistochemistry, *in situ* hybridization, ELISAs and Western blot applications. Especially useful for indirect immunofluorescence, the Alexa Fluor 488 conjugate exhibits excitation and emission maxima that

are similar to fluorescein but the Alexa Fluor 488 dye is brighter, more photostable and its fluorescence is pH insensitive.

Auxiliary Reagents for Use with Anti-Dye and Anti-Hapten Antibodies

Use of the anti-fluorophore, anti-DNP or anti-biotin antibodies requires a means for the selective incorporation of their haptens into proteins, nucleic acids, cells and other biomolecules. Hapten-labeled probes are particularly important for localizing the target in cells and organelles, on blots and in other applications. Detection with an anti-dye antibody permits amplification significantly beyond what is possible with the dye itself. This method can also be used for correlated fluorescence and electron microscopy studies. Anti-fluorophore and anti-biotin antibodies may be particularly useful in combination with the TSA and ELF technologies (Section 6.2, Section 6.3). Molecular Probes has an assortment of reactive haptens, hapten-labeled probes and hapten-labeling kits for these applications, including:

- Chemically reactive haptensylation reagents complementary to each of our anti-dye and anti-hapten antibodies (Section 4.2, Table 4.2)
- ARES and ULYSIS Nucleic Acid Labeling Kits and Alexa Fluor Oligonucleotide Amine Labeling Kits (Section 8.2)
- Protein Labeling Kits for incorporating biotin, biotin/DNP, DSB-X biotin or a wide variety of fluorophores (Section 1.2; Table 1.1, Table 1.2)
- ChromaTide nucleotides — dUTP, OBFA-dCTP or UTP labeled with the fluorescein, Oregon Green 488, Alexa Fluor 488, BODIPY FL, tetramethylrhodamine, Texas Red, Texas Red-X or Cascade Blue fluorophores or the DNP dye (Section 8.2; Table 8.5, Table 8.6)
- Biotin-XX tyramide, DSB-X biotin tyramide, DNP-X tyramide, Oregon Green 488 tyramide and Alexa Fluor 488 tyramide, as components of some of our TSA Kits, for enzyme-amplified staining of cells and tissues (Section 6.2, Table 6.1)
- CellTracker Green CMFDA, CellTracker Orange CMTMR and CellTracker Green BODIPY, derived from fluorescein, tetramethylrhodamine and BODIPY FL dyes, respectively, for cell tracing (Section 14.2)
- LysoTracker Green DND-26 (L-7526) and DAMP (D-1552), which are recognized by our antibodies against the BODIPY FL dye and 2,4-dinitrophenyl (DNP), respectively, for selective staining and ultrastructural localization of intracellular compartments with low pH (Section 12.3, Table 12.6)
- MitoTracker Orange CMTMRos and MitoTracker Red CMXRos, both of which are recognized by the anti-tetramethylrhodamine and anti-Texas Red antibodies, for selective mitochondrial staining (Section 12.2, Table 12.1)
- Biotinylated dextrans, lipids, proteins and microspheres (Section 4.3)
- Polar fluorescent tracers, including derivatives of the Alexa Fluor 488, Oregon Green, Cascade Blue and lucifer yellow dyes and of biotin that can be fixed in cells by aldehyde-based fixatives (Section 14.3)
- Several fluorescent biocytin derivatives (Section 14.3), which permit detection by both avidin-biotin and fluorophore-anti-fluorophore techniques

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Product List — 7.4 Anti-Dye and Anti-Hapten Antibodies

Cat #	Product Name	Unit Size
A-11053	Alexa Fluor® 488 Signal-Amplification Kit for Fluorescein- and Oregon Green® Dye-Conjugated Probes *60–120 assays*	1 kit
A-11094	anti-Alexa Fluor® 488, rabbit IgG fraction *1 mg/mL*	0.5 mL
A-11242	anti-biotin, mouse IgG ₁ , monoclonal 2F5	100 µg
A-11243	anti-biotin, mouse IgG ₁ , monoclonal 2F5, Alexa Fluor® 488 conjugate	100 µg
A-5770	anti-BODIPY® FL, rabbit IgG fraction *3 mg/mL*	0.5 mL
A-5760	anti-Cascade Blue®, rabbit IgG fraction *3 mg/mL*	0.5 mL
A-6398	anti-dansyl, rabbit IgG fraction *1 mg/mL*	0.5 mL
A-6430	anti-dinitrophenyl-KLH, rabbit IgG fraction *2 mg/mL*	0.5 mL
A-11097	anti-dinitrophenyl-KLH, rabbit IgG fraction, Alexa Fluor® 488 conjugate *2 mg/mL*	0.5 mL
A-6435	anti-dinitrophenyl-KLH, rabbit IgG fraction, biotin-XX conjugate *2 mg/mL*	0.5 mL
A-6423	anti-dinitrophenyl-KLH, rabbit IgG fraction, fluorescein conjugate *2 mg/mL*	0.5 mL
A-11095	anti-fluorescein/Oregon Green®, goat IgG fraction *1 mg/mL*	0.5 mL
A-11096	anti-fluorescein/Oregon Green®, goat IgG fraction, Alexa Fluor® 488 conjugate *1 mg/mL*	0.5 mL
A-6421	anti-fluorescein/Oregon Green®, mouse IgG _{2a} , monoclonal 4-4-20	0.5 mg
A-6413	anti-fluorescein/Oregon Green®, rabbit IgG Fab fragment *0.5 mg/mL*	0.5 mL
A-21252	anti-fluorescein/Oregon Green®, rabbit IgG F(ab') ₂ fragment, alkaline phosphatase conjugate	250 µg
A-21254	anti-fluorescein/Oregon Green®, rabbit IgG F(ab') ₂ fragment, horseradish peroxidase conjugate	250 µg
A-889	anti-fluorescein/Oregon Green®, rabbit IgG fraction *1 mg/mL*	0.5 mL
A-11090	anti-fluorescein/Oregon Green®, rabbit IgG fraction, Alexa Fluor® 488 conjugate *1 mg/mL*	0.5 mL
A-11091	anti-fluorescein/Oregon Green®, rabbit IgG fraction, Alexa Fluor® 594 conjugate *1 mg/mL*	0.5 mL
A-21251	anti-fluorescein/Oregon Green®, rabbit IgG fraction, alkaline phosphatase conjugate	0.5 mg
A-982	anti-fluorescein/Oregon Green®, rabbit IgG fraction, biotin-XX conjugate *1 mg/mL*	0.5 mL
A-21253	anti-fluorescein/Oregon Green®, rabbit IgG fraction, horseradish peroxidase conjugate	0.5 mg
A-21250	anti-fluorescein/Oregon Green®, rabbit IgG fraction, R-phycoerythrin conjugate *2 mg/mL*	250 µL
A-5750	anti-lucifer yellow, rabbit IgG fraction *3 mg/mL*	0.5 mL
A-5751	anti-lucifer yellow, rabbit IgG fraction, biotin-XX conjugate *3 mg/mL*	0.5 mL
A-21285	anti-nitrotyrosine, rabbit IgG fraction *1 mg/mL*	0.5 mL
A-6397	anti-tetramethylrhodamine, rabbit IgG fraction *1 mg/mL*	0.5 mL
A-6399	anti-Texas Red®, rabbit IgG fraction *1 mg/mL*	0.5 mL



Coverslip Maxi-Rack

Our unique coverslip maxi-rack (C-24784) provides a simple way to perform experiments that use up to 50 coverslips at one time. The new cover-slip maxi-rack snugly holds 50 coverslips — 18 mm diameter round or 18 mm × 18 mm square — for repeated incubation and washing. The rack can be moved easily between solutions using the metal handle provided, eliminating the need to repeatedly move fragile coverslips with forceps. The maxi-rack is provided with a covered incubation chamber.