

Looking on the bright side with Alexa Fluor® secondary antibody conjugates

Alexa Fluor® 488 dye—a superior alternative to FITC

You have a choice when it comes to green-fluorescent dye conjugates. The Alexa Fluor® 488 dye—with nearly identical spectral properties and quantum yield as fluorescein isothiocyanate (FITC)—produces brighter, more photostable conjugates. These conjugates are ideal for imaging and other applications requiring increased sensitivity and environment-insensitive fluorescence detection.

Seeing green

Since its first synthesis in the 1870s, the fluorescein fluorophore has spread into a multitude of applications, including biological research. The fluorophore has much to recommend it: visible-light excitation and emission, a high quantum yield, and pH sensitivity in the physiological range.

However, as the uses of fluorescein spread and detection technology improved, some limitations began to appear. Although protein conjugates can be made using fluorescein, the fluorophore has a tendency to quench after only a few dye molecules are attached. These conjugates also tend to photobleach quickly, making it difficult to obtain images requiring longer exposure times.

Advantages of the Alexa Fluor® 488 secondary antibody conjugates

Introduced in 1997, the Alexa Fluor® 488 dye is the first dye in 100 years to seriously challenge fluorescein's dominance as the green-fluorescent dye of choice:

- A perfect spectral match for FITC filters—absorption and emission profiles of Alexa Fluor® 488 and fluorescein are nearly identical, so there's no need to change equipment, settings, or filters (Figure 1).
- Brighter conjugate fluorescence—fluorescein conjugates rapidly quench as more fluorophore molecules are added. Alexa Fluor® 488 dye allows more molecules to be attached before self-quenching, producing brighter conjugates (Figure 2).
- Superior photostability—allows more time for image observation and capture, permitting greater sensitivity and simplifying low-abundance target detection (Figure 3).

A cornucopia of Alexa Fluor® 488 dye conjugates

Invitrogen features a wide selection of Alexa Fluor® 488 dye conjugates, including a large selection of secondary antibody conjugates for your most demanding imaging applications. For product details, visit www.invitrogen.com/AF488.

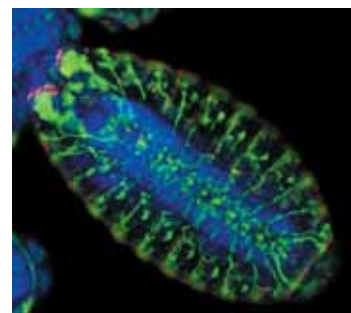


Figure 1—Peripheral nervous system of a wild-type (Canton-S) *Drosophila melanogaster* embryo labeled with the monoclonal 22c10 antibody (which detects a microtubule-associated protein) and subsequently visualized using green-fluorescent Alexa Fluor® 488 rabbit anti-mouse IgG antibody. Dividing cells of the developing denticle bands were labeled with a rabbit anti-histone-H3 antibody and visualized using red-fluorescent Alexa Fluor® 594 goat anti-rabbit IgG antibody. Finally, nuclei, concentrated in the central nervous system, were counterstained with blue-fluorescent DAPI. Image contributed by Neville Cobbe, University of Edinburgh.

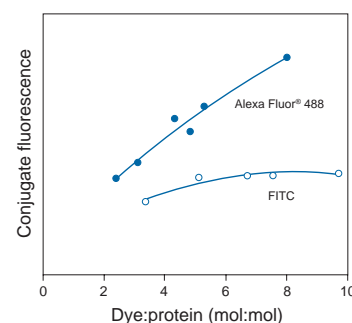


Figure 2—Comparison of the relative fluorescence of goat anti-mouse IgG antibody conjugates prepared from the Alexa Fluor® 488 dye and from fluorescein isothiocyanate (FITC). Conjugate fluorescence is determined by measuring the fluorescence quantum yield of the conjugated dye relative to that of a reference dye and multiplying by the dye:protein labeling ratio.

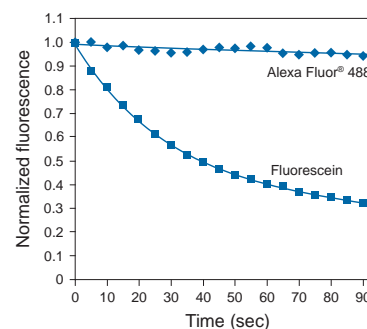
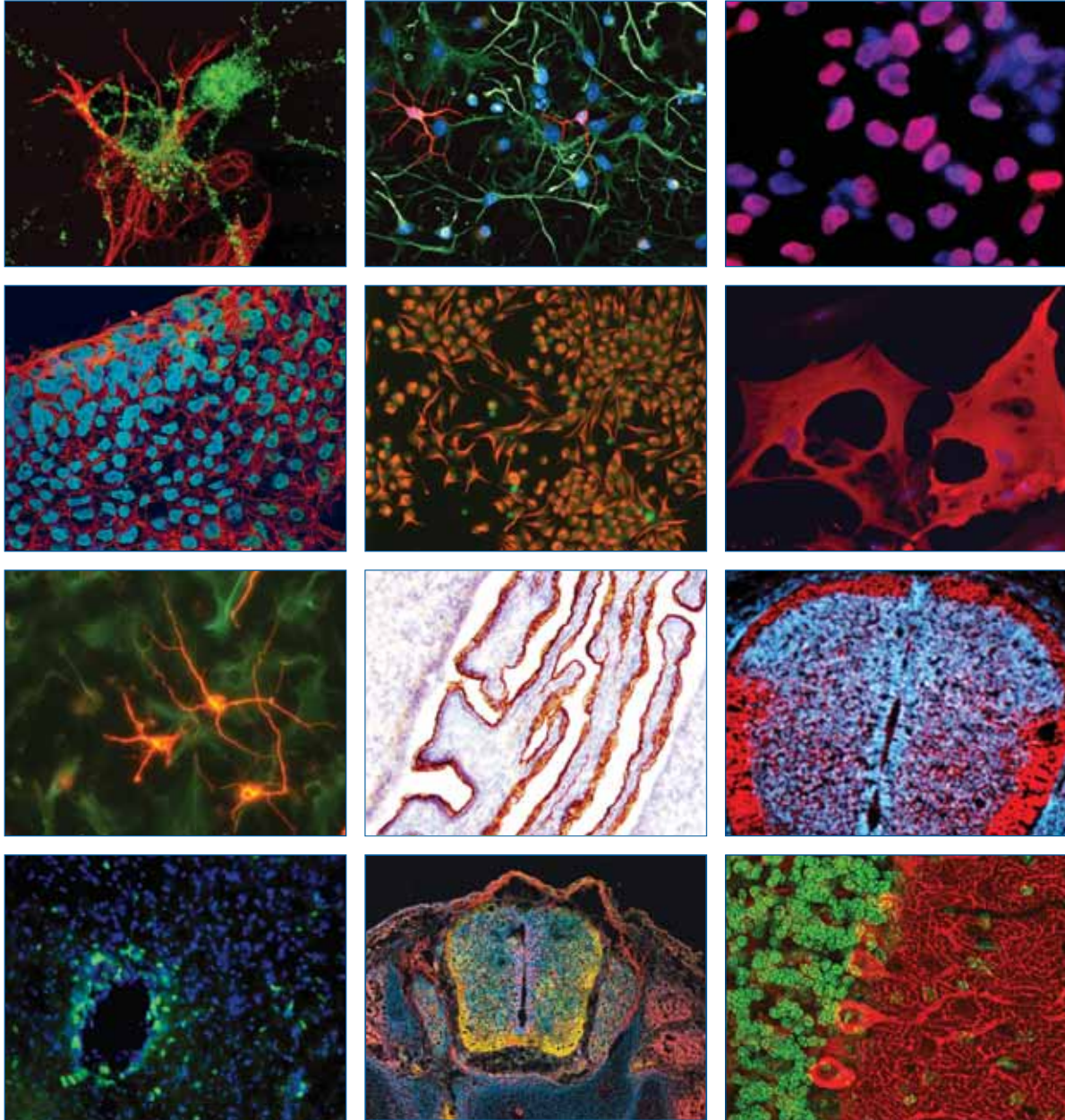


Figure 3—Photobleaching profiles of cells stained with Alexa Fluor® 488 or fluorescein conjugates. Alexa Fluor® 488 or fluorescein conjugates of goat anti-mouse IgG antibody F(ab')₂ fragment were used to detect HEp-2 cells probed with mouse anti-human anti-nuclear antibodies. Samples were continuously illuminated and images were collected every 5 seconds with a cooled CCD camera. Normalized intensity data demonstrate the difference in photobleaching rates.

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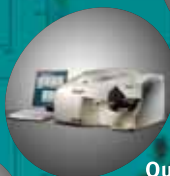
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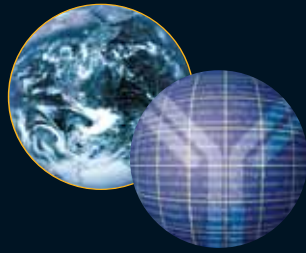
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The National Institute of Allergy and Infectious Diseases (NIAID), Division of Intramural Research (DIR), Laboratory of Virology (LV), Rocky Mountain Laboratories (RML), NIH, DHHS, in Hamilton, Montana, seeks applicants for one or two tenure-track/tenured positions (assistant/associate professor equivalent) to conduct independent research on viral agents requiring high or maximum containment.

The LV conducts high-impact, innovative scientific research on viral agents requiring high or maximum containment, including filoviruses, bunyaviruses, arenaviruses, and flaviviruses with the goal of developing diagnostics, vaccines, and therapeutics. The research conducted in the LV includes studies of vector/reservoir transmission, pathogenesis, pathophysiology and host immune response of high containment viral pathogens. Candidates must be able to develop an independent research program, supervise staff and fellows, and collaborate with RML/DIR researchers working on other viral diseases.

One selected candidate is expected to implement and direct a vigorous, independent research program in molecular virology, antivirals, or vectors and virus transmission of agents requiring high or maximum containment. Fieldwork and ecological studies is a desirable component of these research programs.

The other selected candidate is expected to implement and direct a vigorous, independent research program in host immune responses and/or vaccines against viral agents requiring high or maximum containment. This program is expected to include studies of innate and adaptive immune responses in animal models.

Candidates for either position must hold a Ph.D., D.V.M., or M.D. degree and have a minimum of 3 years of relevant postdoctoral experience. Independent resources including space, support personnel, and an annual budget for services, supplies, and salaries are committed to the positions. Facilities at existing NIAID field sites in Africa and Asia may be available to the incumbents, and support for field work and ecological studies at new sites is possible. These are appointments under Title 42. Salary is dependent on experience and qualifications.

RML's state-of-the-art facilities include an operational BSL-3 facility, a BSL-4 laboratory and animal facility nearing completion that can accommodate work with both small animal and non-human primate models, and core facilities for genomics, electron microscopy, and flow cytometry. Other RML research programs focus on prions, retroviruses, numerous pathogenic prokaryotic organisms and pathogen transmission by arthropod vectors. RML is located in the scenic Bitterroot Valley of western Montana within easy access to some of the finest outdoor recreational opportunities in North America. Additional information on the positions can be obtained by contacting Dr. Marshall E. Bloom at mbloom@niaid.nih.gov.

Application Process: To apply, submit a curriculum vitae and bibliography, including a list of your five most significant papers and a 2-3-page description of a proposed research program via e-mail to Wanda Jackson at NIAID.DIR.Search@niaid.nih.gov. In addition, three letters of recommendation must be sent directly from the referees to Dr. Jeffery Taubenberger, Chair, NIAID Search Committee, c/o Wanda Jackson at NIAID.DIR.Search@niaid.nih.gov or 10 Center Drive, MSC 1356, Building 10, Room 4A22, Bethesda, Maryland 20892-1356. E-mail is preferred. Completed applications will be reviewed starting **September 1, 2008**. Please refer to ad #023 on all correspondence. Further information regarding LV is available at <http://www3.niaid.nih.gov/labs/aboutlabs/LV/>, information regarding the DIR laboratories is available at <http://www3.niaid.nih.gov/about/organization/dir/default.htm> and information about working at NIAID is available at <http://healthresearch.niaid.nih.gov>.

Applicants must be U.S. citizens, resident aliens, or nonresident aliens with or eligible to obtain a valid employment-authorizing visa.



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