Alexa Fluor® SFX Kits

Quick Facts

Storage upon receipt:

- Component A: ≤6ºC; AVOID FREEZE-THAW; protect from light
- Component B: ≤25ºC

Ex/Em: See Table 1

Introduction

Alexa Fluor® SFX Kits provide the ultimate in convenient, reliable small-scale antibody labeling with consistently high-quality results. These kits provide either goat anti–mouse IgG or goat anti–rabbit IgG secondary antibody (as standard or highly cross-adsorbed preparations) conjugated to Alexa Fluor® 488, Alexa Fluor® 555, Alexa Fluor® 594, or Alexa Fluor® 647, four of our most popular Alexa Fluor® dyes (Table 1).

The Alexa Fluor® SFX Kits further optimize your results by employing our Image-iT™ FX signal enhancer. This product dramatically improves the signal-to-noise ratio of immunolabeled cells and tissues by efficiently blocking nonspecific interactions of a wide variety of fluorescent dyes with cell and tissue constituents (Table 2, Figure 1). Targets that are usually indistinguishable or blurry due to background fluorescence can be clearly visualized by including this background suppressor in the staining protocol.

In addition to the antibodies included in the Alexa Fluor® SFX Kits, Molecular Probes prepares conjugates of other species-specific anti-IgG antibodies, with many other Alexa Fluor® and other fluorescent labels, as well as conjugates of biotin, avidin, streptavidin, NeutrAvidin™ biotin-binding protein, protein A, and protein G. Please visit our website at probes.invitrogen.com, or contact our Technical Services Department for more information about these products.

IgG Antibody Conjugates

Molecular Probes goat anti–mouse IgG conjugates are prepared from affinity-purified antibodies that react with IgG heavy chains and all classes of immunoglobulin light chains from mouse. To minimize cross-reactivity, the goat anti–mouse IgG whole antibodies have been adsorbed against human IgG and human serum prior to conjugation.

Molecular Probes goat anti–rabbit IgG conjugates are prepared from affinity-purified antibodies that react with IgG heavy chains and all classes of immunoglobulin light chains from rabbit. To minimize cross-reactivity, the goat anti–rabbit IgG whole

Table 1. Alexa Fluor® SFX Kits.

Kit	Ex *	Em *	Standard antibody †	Highly cross-adsorbed ‡
Alexa Fluor® 488 Goat Anti-Mouse SFX Kit	495	519	A31619	A31620
Alexa Fluor® 488 Goat Anti-Rabbit SFX Kit	495	519	A31627	A31628
Alexa Fluor® 555 Goat Anti-Mouse SFX Kit	555	565	A31621	A31622
Alexa Fluor® 555 Goat Anti-Rabbit SFX Kit	555	565	A31629	A31630
Alexa Fluor® 594 Goat Anti-Mouse SFX Kit	590	617	A31623	A31624
Alexa Fluor® 594 Goat Anti-Rabbit SFX Kit	590	617	A31631	A31632
Alexa Fluor® 647 Goat Anti-Mouse SFX Kit §	650	668	A31625	A31626
Alexa Fluor® 647 Goat Anti-Rabbit SFX Kit §	650	668	A31633	A31634

***** Approximate fluorescence excitation (Ex) and emission (Em) maxima, in nm, for conjugates. Complete spectra for these dyes are available at our website (probes.invitrogen.com). **†** Goat anti-mouse whole antibodies have been cross-adsorbed against human IgG and human serum. Goat anti-rabbit whole antibodies have been cross-adsorbed against human IgG, human serum, mouse IgG, mouse serum, and bovine serum. **‡** Goat anti-mouse whole antibodies have been cross-adsorbed against bovine IgG, goat IgG, rabbit IgG, human IgG and serum, and rat IgG. Goat anti-rabbit whole antibodies have been cross-adsorbed against bovine IgG, goat IgG, human IgG, mouse IgG, and rat IgG. **§** Human vision is insensitive to light beyond ~650 nm, and therefore it is not possible to view the fluorescence of these dyes by looking through a conventional fluorescence microscope.

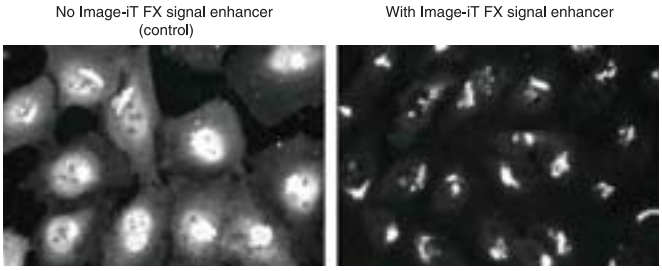

Figure 1. Effect of Image-iT™ FX signal enhancer. Fixed and permeabilized HeLa cells were incubated with 1% (w/v) BSA, and Image-iT™ FX signal enhancer was applied. The cells were then stained using a mouse monoclonal anti–golgin 97 primary antibody, followed by Alexa Fluor® 488 goat anti–mouse IgG (H+L) (A11001). Cells not treated with Image-iT™ FX signal enhancer show strong nuclear and cytoplasmic background staining in addition to the specific Golgi staining, whereas cells treated with Image-iT™ FX signal enhancer show only the specific Golgi staining. Image-iT™ FX signal enhancer can also be used after staining with fluorescent primary and secondary antibodies and other fluorescent bioconjugates to block background staining and enhance the specific signal.

antibodies have been adsorbed against human IgG, human serum, mouse IgG, mouse serum, and bovine serum prior to conjugation.

Highly Cross-Adsorbed IgG Antibody Conjugates

For researchers interested in highly cross-adsorbed antibodies, the Alexa Fluor® SFX Kits are available with goat anti–mouse IgG whole antibodies that have been adsorbed against bovine, goat, rabbit, rat, and human IgGs, and human serum; and goat anti–rabbit IgG whole antibodies that have been adsorbed against bovine, goat, mouse, rat, and human IgGs. These highly cross-adsorbed antibodies may be useful in multilabeling experiments or for labeling cells or tissues where nonspecific staining has been a problem. Because our highly cross-adsorbed goat anti–mouse IgG antibodies have been adsorbed against rat IgG, they are particularly useful for detecting mouse IgG in rat tissues or cells and in experiments in which mouse antibodies are being detected in the presence of rat antibodies. Please note, however, that because rats and mice are closely related, the adsorption against rat IgG may have reduced the specificity of the goat anti–mouse IgG antibody preparations for certain mouse IgG subclasses.

Image-iT™ FX Signal Enhancer

Image-iT™ FX signal enhancer is a unique and highly effective product for blocking background staining that results from nonspecific interactions of a wide variety of fluorescent dyes with cell and tissue constituents (Table 2). Background fluorescence is largely eliminated when Image-iT™ FX signal enhancer is applied to fixed and permeabilized cells prior to staining with fluorescent antibody conjugates. (**Note:** Image-iT™ FX signal enhancer is not intended for use with live cells.) The signal enhancer may also effectively prevent nonspecific staining that is typically blocked with 1–2% BSA or 10% serum treatment, thus eliminating the need for another step in the staining protocol in some cases.

Table 2. Fluorescent Dyes Successfully Tested with the Image-iT™ FX Signal Enhancer.*

Dyes with Potentially Strong Background Fluorescence That Is Reduced with the Image-iT™ FX Signal Enhancer †		
Fluorescein	Atto 610	Alexa Fluor® 610
Oregon Green® 488	Cascade Blue®	Alexa Fluor® 633
Oregon Green® 514	Alexa Fluor® 405	Alexa Fluor® 635
Tetramethylrhodamine	Alexa Fluor® 430	Alexa Fluor® 647
Texas Red®	Alexa Fluor® 488	Alexa Fluor® 660
Cascade Yellow™	Alexa Fluor® 514	Alexa Fluor® 680
Dy 565	Alexa Fluor® 555	Alexa Fluor® 700
Dy 630	Alexa Fluor® 568	Alexa Fluor® 750
Atto 590	Alexa Fluor® 594	
Dyes with Potentially Weak Background Fluorescence That Is Reduced with the Image-iT™ FX Signal Enhancer †		
Cy5	Alexa Fluor® 546	DyeMer™ 488/605
Dy 635	Allophycocyanin	DyeMer™ 488/615
Marina Blue®	R-phycoerythrin	DyeMer™ 488/630
Alexa Fluor® 532		
Background-Free Fluorescent Dyes That Are Not Affected by the Image-iT™ FX Signal Enhancer ‡		
Alexa Fluor® 350	Pacific Blue™	Texas Red®-X
IRTM 790	Rhodamine B	Dy 550
Cy3	Rhodamine Red™-X	Dy 610

* All dyes were conjugated to streptavidin and tested at 10 µg/mL. † Background staining was blocked by the Image-iT™ FX signal enhancer. ‡ Staining was unaffected by the Image-iT™ FX signal enhancer.

Materials

- **Alexa Fluor® dye–labeled IgG (H+L) secondary antibody**, 2 mg/mL, 400 µg (Component A)
- **Image-iT™ FX signal enhancer**, 1X, 10 mL (Component B)

The components in the Alexa Fluor® SFX Kits are available as stand-alone products. The dye-labeled secondary antibodies are offered in unit sizes of 0.5 mL of 2 mg/mL solution (1 mg). Image-iT™ FX signal enhancer is offered in the 10 mL unit size. Please see the Product List for ordering information.

Fluorophore-Labeled Antibodies

Alexa Fluor® dye–labeled antibodies, including highly cross-adsorbed antibodies, are supplied in the Alexa Fluor® SFX Kits as 2 mg/mL solutions in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, 5 mM sodium azide. When these products are stored undiluted at 2–6°C, they are stable for at least three months. For longer storage, divide the solution into single-use aliquots and freeze at ≤–20°C. Frozen aliquots are stable for at least six months.

The degree of labeling for each conjugate is typically 2–8 fluorophore molecules per IgG molecule; the exact degree of labeling is indicated on the product label. At the time of preparation, the products are certified to be free of unconjugated dyes and are tested in a cytological experiment to ensure low nonspecific staining. PROTECT FLUORESCENT CONJUGATES FROM LIGHT. AVOID REPEATED FREEZING AND THAWING.

Image-iT™ FX Signal Enhancer

The Image-iT™ FX signal enhancer is supplied as a 1X solution in phosphate-buffered saline (PBS, pH 7.2) containing 2 mM sodium azide as a preservative. The reagent comes in a plastic dropper bottle and can be applied directly to cells or tissues without further dilution. Each bottle is sufficient for at least 50 coverslip-sized experiments using the protocol described below. The Image-iT™ FX signal enhancer can be stored at ≤25°C (including ≤–20°C) and is stable for at least 6 months.

Procedures

General Fixation Protocol

This is a typical protocol for fixing cells prior to incubation with primary and secondary antibodies. This protocol should be used as a general guideline and may require further optimization for your specific application.

1.1 Rinse the cells in buffer (Hanks' Balanced Salt Solution (HBSS), phosphate-buffered saline (PBS), or Tyrode's-HEPES) at 37°C to remove culture media. Keep the buffer warm to prevent heat-shock and detachment of the cells.

1.2 Fix the samples in warm (37°C) 3.7% formaldehyde (diluted in buffer); incubate 10–15 minutes at room temperature. High-quality formaldehyde is important for good results—we recommend Polysciences, Inc. catalog #18814 (16% formaldehyde,

methanol free, ultrapure). Slightly longer fixation times (20–30 minutes) may be acceptable if they do not disrupt the immunoreactivity of the target(s).

1.3 Rinse the samples in buffer 3–4 times for one minute per rinse. Cells grown on coverslips can be rinsed through several beakers of buffer for 15–20 seconds per rinse.

1.4 Permeabilize the cells in 0.2% TRITON X-100 (diluted in buffer) for 5 minutes or in 0.1% TRITON X-100 for 15 minutes.

1.5 Rinse the samples 3–4 times in buffer.

Protocol for Blocking with Image-iT™ FX Signal Enhancer

2.1 Fix and permeabilize the cells or tissue sections using either the procedure described under General Fixation Protocol or your own procedure. Image-iT™ FX signal enhancer is not intended for use with live cells.

2.2 Rinse the samples with buffer.

2.3 Apply 4 drops (~200 µL) of Image-iT™ FX signal enhancer or sufficient volume to cover each coverslip or section. Incubate for 30 minutes at room temperature in a humid environment.

2.4 Rinse thoroughly with buffer.

2.5 Proceed with the normal staining protocol. The Image-iT™ FX signal enhancer will not be displaced during subsequent wash steps. Note that it is not necessary to perform the 10% serum or 1–2% BSA blocking steps commonly associated with antibody staining protocols, although additional blocking steps may be performed subsequent to blocking with the Image-iT™ solution, if desired. However, do not add serum or BSA directly to the Image-iT™ FX signal enhancer, as they may reduce the effectiveness of this product.

Antibody Staining

It is good practice to centrifuge the labeled antibody solution briefly in a microcentrifuge before use; only the supernatant should then be used in the experiment. This step will eliminate any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining.

Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. For Alexa Fluor® dye–labeled antibodies, a final concentration of 1–10 µg/mL should be satisfactory for most immunohistochemical applications.[1]

Reference

1. Short Protocols in Molecular Biology, 2nd Edition, F.M. Ausubel et al., Eds., John Wiley and Sons (1992) pp. 14-24–14-30.

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

Cat #	Product Name	Unit Size
A31619	Alexa Fluor® 488 Goat Anti-Mouse SFX Kit	1 kit
A31620	Alexa Fluor® 488 Goat Anti-Mouse SFX Kit *highly cross-adsorbed*	1 kit
A31621	Alexa Fluor® 555 Goat Anti-Mouse SFX Kit	1 kit
A31622	Alexa Fluor® 555 Goat Anti-Mouse SFX Kit *highly cross-adsorbed*	1 kit
A31623	Alexa Fluor® 594 Goat Anti-Mouse SFX Kit	1 kit
A31624	Alexa Fluor® 594 Goat Anti-Mouse SFX Kit *highly cross-adsorbed*	1 kit
A31625	Alexa Fluor® 647 Goat Anti-Mouse SFX Kit	1 kit
A31626	Alexa Fluor® 647 Goat Anti-Mouse SFX Kit *highly cross-adsorbed*	1 kit
A31627	Alexa Fluor® 488 Goat Anti-Rabbit SFX Kit	1 kit
A31628	Alexa Fluor® 488 Goat Anti-Rabbit SFX Kit *highly cross-adsorbed*	1 kit
A31629	Alexa Fluor® 555 Goat Anti-Rabbit SFX Kit	1 kit
A31630	Alexa Fluor® 555 Goat Anti-Rabbit SFX Kit *highly cross-adsorbed*	1 kit
A31631	Alexa Fluor® 594 Goat Anti-Rabbit SFX Kit	1 kit
A31632	Alexa Fluor® 594 Goat Anti-Rabbit SFX Kit *highly cross-adsorbed*	1 kit
A31633	Alexa Fluor® 647 Goat Anti-Rabbit SFX Kit	1 kit
A31634	Alexa Fluor® 647 Goat Anti-Rabbit SFX Kit *highly cross-adsorbed*	1 kit
I36933	Image-iT™ FX signal enhancer	10 mL
A11001	Alexa Fluor® 488 goat anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A11029	Alexa Fluor® 488 goat anti-mouse IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21422	Alexa Fluor® 555 goat anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A21424	Alexa Fluor® 555 goat anti-mouse IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A11005	Alexa Fluor® 594 goat anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A11032	Alexa Fluor® 594 goat anti-mouse IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21235	Alexa Fluor® 647 goat anti-mouse IgG (H+L) *2 mg/mL*	0.5 mL
A21236	Alexa Fluor® 647 goat anti-mouse IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A11008	Alexa Fluor® 488 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11034	Alexa Fluor® 488 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21428	Alexa Fluor® 555 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21429	Alexa Fluor® 555 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A11012	Alexa Fluor® 594 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A11037	Alexa Fluor® 594 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL
A21244	Alexa Fluor® 647 goat anti-rabbit IgG (H+L) *2 mg/mL*	0.5 mL
A21245	Alexa Fluor® 647 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* *2 mg/mL*	0.5 mL

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Please visit our website—**probes.invitrogen.com**—for the most up-to-date information.

Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 • Fax: (541) 335-0504

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

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

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

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