

## Technical Tip: Eliminate Background Staining with the Image-iT™ FX Signal Enhancer

The Image-iT™ FX signal enhancer (I36933) 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 (Figure 1). Targets that are usually indistinguishable or blurry due to background fluorescence can now be clearly visualized by including this background suppressor in the staining protocol.

Table 1 lists the fluorescent dyes whose protein conjugates have been successfully tested with the Image-iT™ FX signal

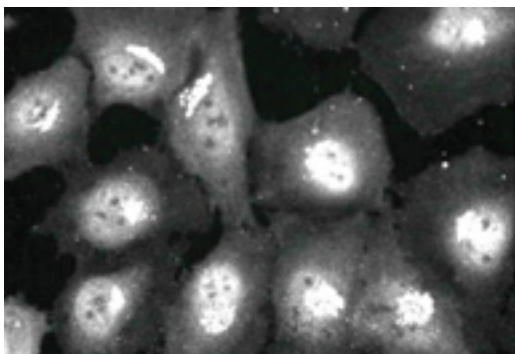
enhancer. Background staining is largely eliminated when the Image-iT™ FX signal enhancer is applied to fixed and permeabilized cells prior to staining (this reagent is not intended for use with live cells). This reagent comes ready-to-use as a 10 ml solution—a sufficient volume for at least 50 coverslip-size experiments using the protocol provided. Simply add 4 drops of the enhancer, incubate, and proceed with your normal blocking step.

**Table 1.** Fluorescent Dyes Successfully Tested with the Image-iT™ FX Signal Enhancer.\*

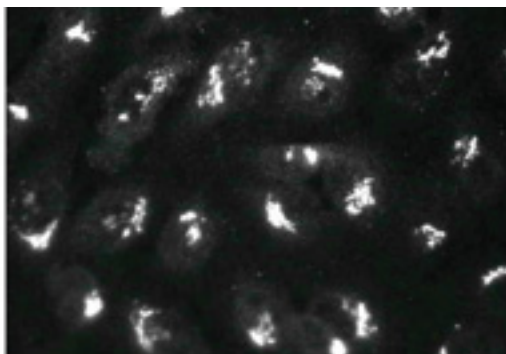
<b>Dyes with Potentially Strong Background Fluorescence that is Reduced with the Image-iT™ FX Signal Enhancer †</b>		
Fluorescein	Atto 610	Alexa Fluor® 594
Oregon Green® 488	Cascade Blue®	Alexa Fluor® 610
Oregon Green® 514	Alexa Fluor® 405	Alexa Fluor® 633
Tetramethylrhodamine	Alexa Fluor® 430	Alexa Fluor® 635
Texas Red®	Alexa Fluor® 488	Alexa Fluor® 647
Cascade Yellow™	Alexa Fluor® 500	Alexa Fluor® 660
Dy 565	Alexa Fluor® 514	Alexa Fluor® 680
Dy 630	Alexa Fluor® 555	Alexa Fluor® 700
Atto 590	Alexa Fluor® 568	Alexa Fluor® 750
<b>Dyes with Potentially Weak Background Fluorescence that is Reduced with the Image-iT™ FX Signal Enhancer †</b>		
Cy5	Alexa Fluor® 546	DyeMer™ 488/605
Dy 635	Allophycocyanin	DyeMer™ 488/615
Marina Blue®	R-phycoerythrin	DyeMer™ 488/630
Alexa Fluor® 532		
<b>Background-Free Fluorescent Dyes that are not Affected by the Image-iT™ FX Signal Enhancer ‡</b>		
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.

No Image-iT FX signal enhancer  
(control)



With Image-iT FX signal enhancer



**Figure 1.** HeLa cells were washed with PBS, and fixed cells were then rinsed with PBS and permeabilized. After additional rinsing with PBS, the cells were incubated for 30 minutes at room temperature with 1% (w/v) bovine serum albumin in PBS. The cells were rinsed again with PBS, and Image-iT™ FX Signal Enhancer (I36933) was used as described in the instructions accompanying the product. Control cells were incubated with PBS alone. The cells were then stained by incubating them with a mouse monoclonal anti-golgin 97 primary antibody (A21270) at 0.5 µg/mL in PBS for 1 hour at room temperature. After rinsing with PBS, the cells were incubated with a fluorescent secondary antibody conjugate, goat anti-mouse IgG, Alexa Fluor 488 (A11001) at 5 µg/mL in PBS for 1 hour at room temperature. Finally, the cells were washed with PBS, mounted, and imaged by fluorescence microscopy.

Control cells not treated with Image-iT™ FX signal enhancer showed strong nuclear and cytoplasmic background staining in addition to the specific Golgi staining. Cells treated with Image-iT™ FX signal enhancer showed only the specific Golgi staining. Similar results were obtained when Image-iT™ FX signal enhancer was mixed with either the primary or the secondary antibody, but the signal enhancer was most effective when it was used separately as described above. 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.

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