Image-iT™ FX Signal Enhancer

Catalog no. I36933

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage*	Stability
Image-iT™ FX signal enhancer*	10 mL	1X solution in phosphate-buffered saline (PBS, pH 7.2) containing 2 mM sodium azide as a preservative	• ≤25˚C	When stored as directed this kit is stable for at least 6 months
Number of assays: Sufficient material is supplied for at least 50 coverslip-sized experiments using the protocol described below.				
***Image-iT™ FX signal enhancer is not intended for use with live cells.**				

Introduction

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. Background staining seen with fluorescent conjugates of streptavidin, goat anti–mouse IgG, or goat anti–rabbit IgG is largely eliminated when the Image-iT™ FX signal enhancer is applied to fixed and permeabilized cells prior to staining. Table 2 lists the fluorescent dye conjugates that have been successfully tested with the Image-iT™ FX signal enhancer.

Before Starting

Materials Required but Not Provided

- Hanks' Balanced Salt Solution (HBSS), phosphate-buffered saline (PBS), or Tyrodes-HEPES
- 3.7% formaldehyde (diluted in buffer); Polysciences Cat. no. 18814 (16% ultrapure formaldehyde, methanol free)
- 0.2% Triton® X-100 (diluted in buffer)

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

Fluorescent dyes		
Alexa Fluor® 350	Alexa Fluor® 633	Cascade Blue®
Alexa Fluor® 405	Alexa Fluor® 635	Pacific Blue™
Alexa Fluor® 430	Alexa Fluor® 647	Fluorescein
Alexa Fluor® 488	Alexa Fluor® 660	Oregon Green® 488
Alexa Fluor® 532	Alexa Fluor® 680	Tetramethylrhodamine
Alexa Fluor® 546	Alexa Fluor® 700	Cy3
Alexa Fluor® 555	Alexa Fluor® 750	Rhodamine B
Alexa Fluor® 568		Rhodamine Red-X
Alexa Fluor® 594		Texas Red®, Texas Red-X
Alexa Fluor® 610		Cy5
*All dyes were conjugated to streptavidin and tested at 10 µg/mL.		

Experimental Protocols

General Fixation Protocol

Below is a typical protocol for fixing cells prior to incubation with primary and secondary antibodies. Use this protocol 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 Tyrodes-HEPES) at 37°C to remove culture media. Keep the buffer warm to prevent heat shocking and detachment of the cells.

1.2 Fix the samples in warm (37°C) 3.7% formaldehyde in buffer; incubate for 10–15 minutes at room temperature. 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 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

The Image-iT™ FX signal enhancer is supplied ready-to-use in a plastic dropper bottle and can be applied directly to cells or tissues without further dilution.

2.1 Fix and permeabilize the cells or tissue sections using the procedure described under *General Fixation Protocol* or your own procedure.

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. Additional blocking steps may be performed subsequent to blocking with the Image-iT™ signal enhancer, as needed. Do not add serum or BSA directly to the Image-iT™ FX signal enhancer, as they can reduce the effectiveness of this product.

References

1. Molecular Biomethods Handbook, Humana Press, 631 (1998); **2.** J Histochem Cytochem 51, 1669 (2003).

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

Cat. no.	Product Name	Unit Size
I36933	Image-iT™ FX signal enhancer	10 mL

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