

# Image-iT<sup>®</sup> FX Signal Enhancer



- 10 ml
- Sample Size

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**For Research Use Only. Not For Use In Diagnostic Procedures.**

**Applications**  
IF-F, IF-IC, IF-P

**Species Cross-Reactivity**  
All

## Fluorescent Dyes Successfully Tested with the Image-iT<sup>®</sup> FX Signal Enhancer

### Dyes with Potentially Strong Background Fluorescence<sup>1</sup>

- Fluorescein
- Oregon Green<sup>®</sup> 488 & 514
- Tetramethylrhodamine
- Texas Red<sup>®</sup>
- Cascade Yellow<sup>™</sup>
- Dy 565 & 630
- Atto 590 & 610
- Alexa Fluor<sup>®</sup> 405, 430, 488, 500, 514, 555, 568, 594, 610, 633, 635, 647, 660, 680, 700, & 750
- Cascade Blue<sup>®</sup>

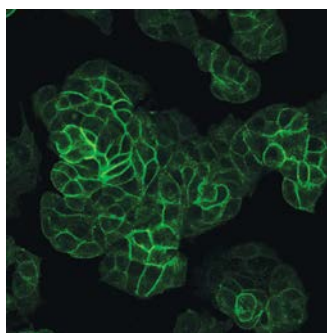
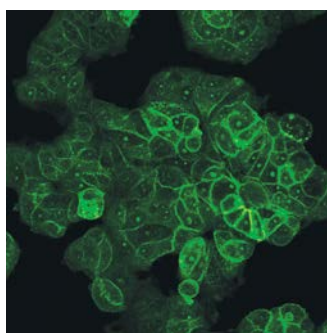
### Dyes with Potentially Weak Background Fluorescence<sup>1</sup>

- Cy 5
- Dy 635
- Marina Blue<sup>®</sup>
- Alexa Fluor<sup>®</sup> 532 & 546
- Allophycocyanin
- R-phycoerythrin
- DyeMer<sup>™</sup> 488/605, 488/615, & 488/630

### Background-Free Fluorescent Dyes<sup>2</sup>

- Alexa Fluor<sup>®</sup> 350
- IRTM 790
- Cy 3
- Pacific Blue<sup>™</sup>
- Rhodamine B
- Rhodamine Red<sup>™</sup>-X
- Texas Red<sup>®</sup>-X
- Dy 550 & 610

**Description:** Alexa Fluor<sup>®</sup> and many other anionic fluorescent dyes and proteins can bind nonspecifically with cationic cell and tissue constituents. By efficiently blocking these nonspecific electrostatic interactions, Image-iT<sup>®</sup> FX Signal Enhancer can dramatically improve the signal-to-noise ratio of immunolabeled cells and tissues. Image-iT<sup>®</sup> is a liquid that is applied directly to slides or coverslips containing fixed and permeabilized cell or tissue samples prior to staining with fluorescent probes.



Confocal immunofluorescent analysis of MCF7 cells using EpCAM (D1B3) Rabbit mAb #2626 (green) either untreated (upper), or treated with Image-iT<sup>®</sup> FX Signal Enhancer (lower). Note the reduction in non-specific, nucleolar background following pre-treatment with Image-iT<sup>®</sup> FX Signal Enhancer.

**Storage:** Store at room temperature. This product is stable for 12 months.

**Directions for Use:** Following specimen preparation:

1. Permeabilize the cells in Permeabilization Buffer for 5 minutes at room temperature.
2. Rinse three times in PBS for 5 minutes each.
3. Apply 3–4 drops of Image-iT<sup>®</sup> FX Signal Enhancer (or sufficient volume to cover the cells) and incubate for 30 minutes at room temperature.
4. Rinse three times with PBS for 5 minutes each.
5. Block specimen in Blocking Buffer for 60 minutes.
6. While blocking, prepare primary antibody by diluting as indicated on datasheet in Antibody Dilution Buffer.
7. Aspirate blocking solution, apply diluted primary antibody.
8. Incubate overnight at 4°C.
9. Rinse three times in PBS for 5 minutes each.
10. Coverslip slides with Prolong<sup>®</sup> Gold Antifade Reagent.
11. For best results, examine specimens immediately using appropriate excitation wavelength. For long-term storage, store slides flat at 4°C protected from light.

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