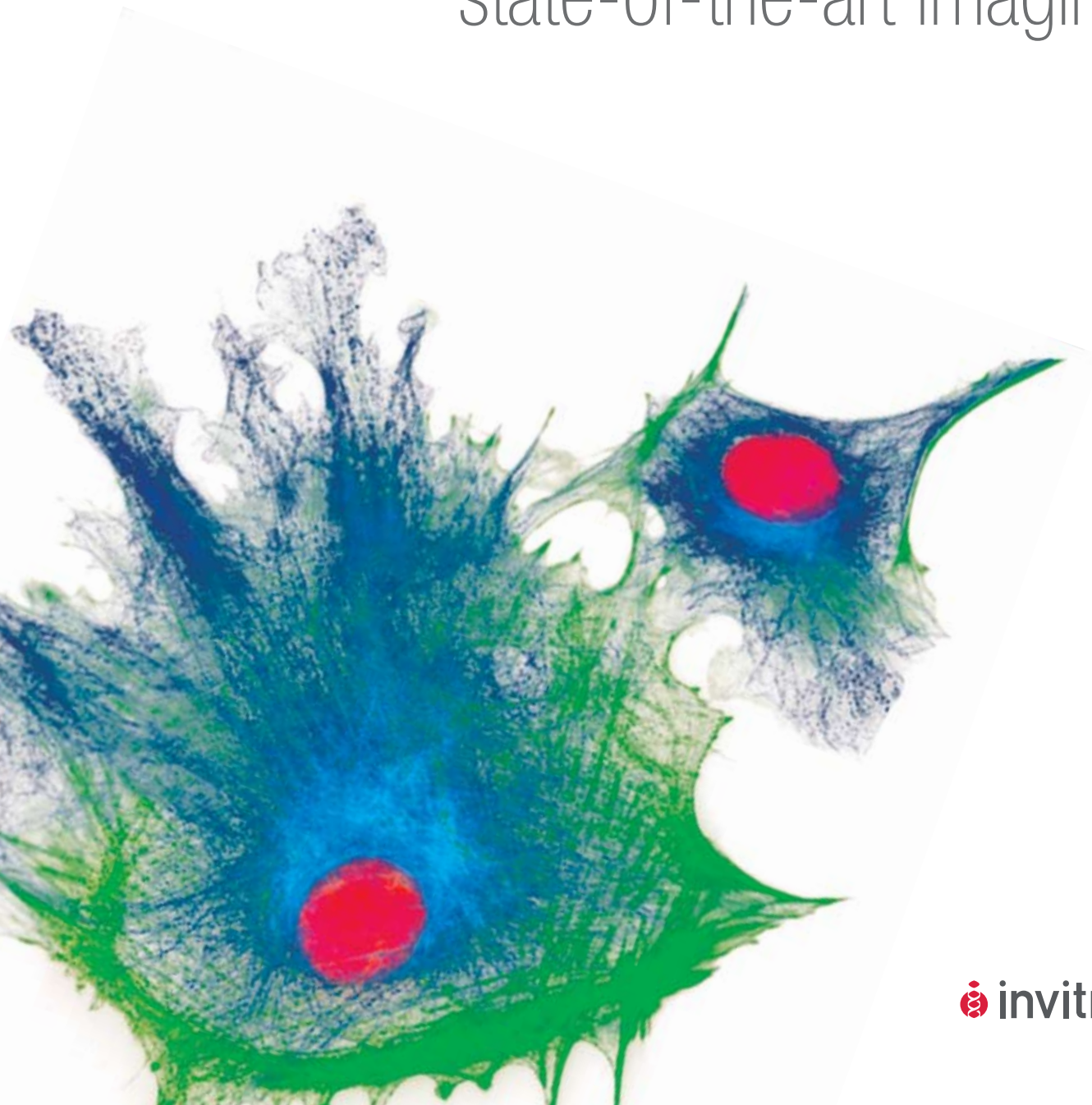




# Products for state-of-the-art imaging





# Products for state-of-the-art imaging

- Detect your targets with the brightest secondary antibody conjugates
- Improve signal-to-noise ratio and reduce photobleaching
- Get unsurpassed imaging results with imaging kits
- Ensure exceptional optical performance

Invitrogen offers you the best Molecular Probes™ products and years of experience to help you get the most from your imaging experiments. These include the brightest and most photostable fluorescent dyes and dye conjugates, the most innovative reagents and kits for enhanced imaging performance, and a broad range of superior filters and imaging accessories.

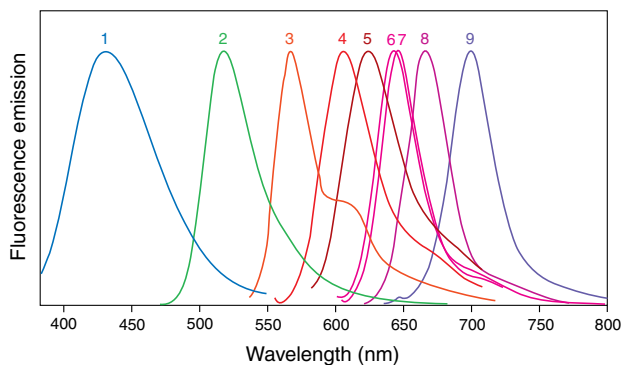
## Detect your targets with the brightest secondary antibody conjugates

### The Alexa Fluor® dye series

The Alexa Fluor® dyes—a series of superior fluorescent dyes that spans the near-UV, visible, and near-IR range—represent a

major breakthrough in the development of fluorescent labeling reagents. These dyes, without exception, produce the best and brightest conjugates we have ever tested, and provide many options for multicolor detection and fluorescence resonance energy transfer (FRET). Benefits of the Alexa Fluor® dyes and their conjugates include:

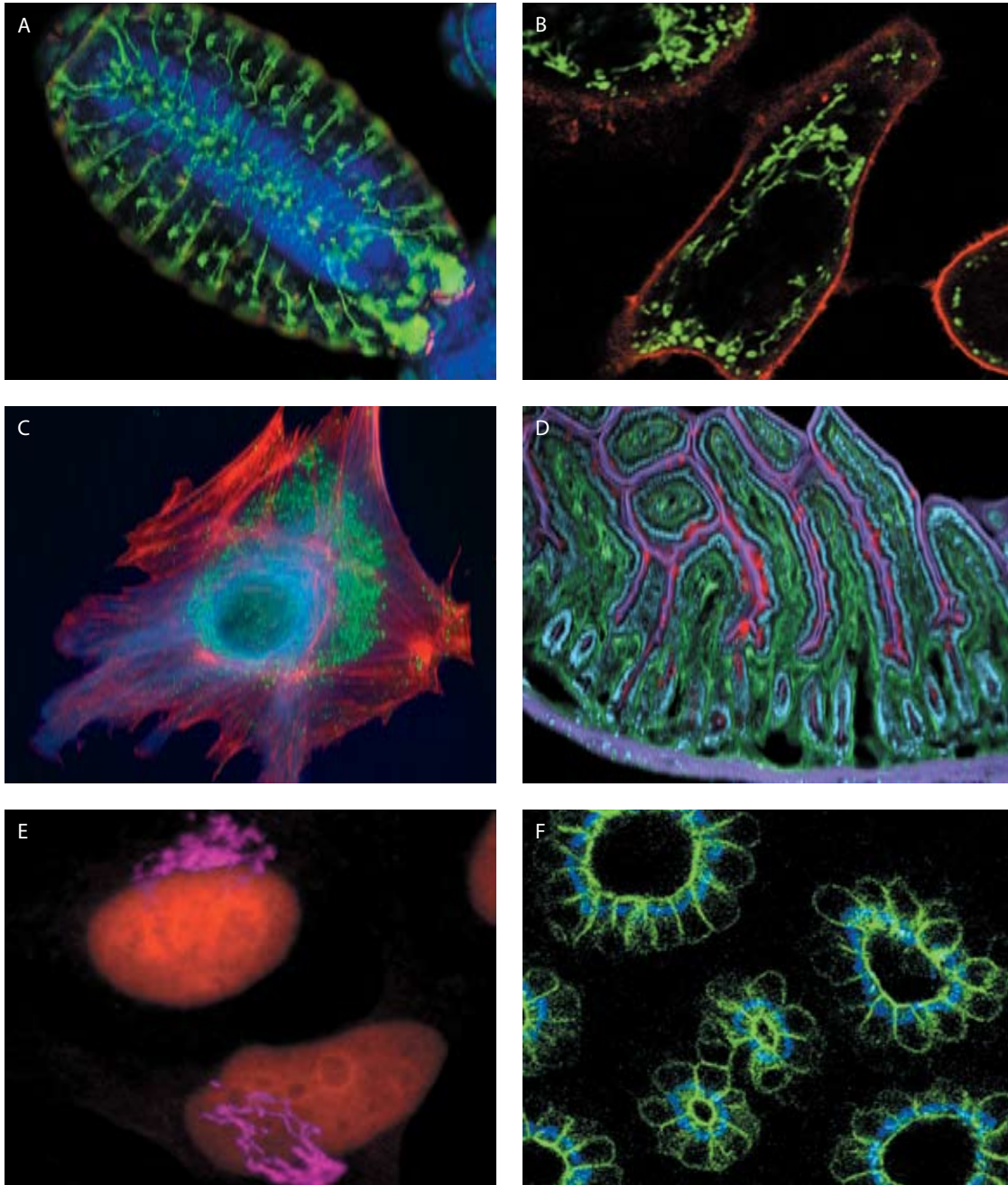
- Color selection—Alexa Fluor® conjugates are available in an array of distinct fluorescent colors from blue to near infrared (Figures 1 and 2)



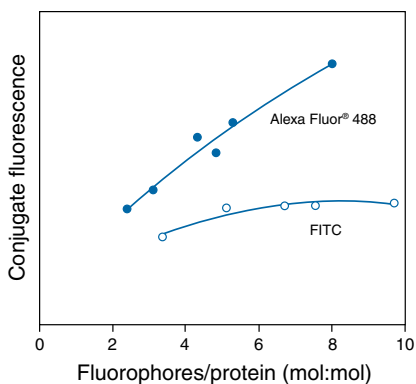
Color	Alexa Fluor® dye	Abs*	Em*	Extinction coefficient †
1	Alexa Fluor® 350	346	442	19,000
2	Alexa Fluor® 488	495	519	71,000
3	Alexa Fluor® 555	555	565	150,000
4	Alexa Fluor® 568	578	603	91,300
5	Alexa Fluor® 594	590	617	73,000
6	Alexa Fluor® 633	632	647 ‡	100,000
7	Alexa Fluor® 635	633	647 ‡	140,000
8	Alexa Fluor® 647	650	665 ‡	239,000
9	Alexa Fluor® 680	679	702 ‡	184,000

\* Absorption and fluorescence emission maxima, in nm. † Extinction coefficient at  $\lambda_{max}$  in  $cm^2 M^{-1}$ . ‡ Human vision is insensitive to light beyond ~650 nm, and therefore it is not possible to view the far-red-fluorescent dyes by looking through the eyepiece of a conventional fluorescent microscope. Colors in this table correspond to the spectra at left.

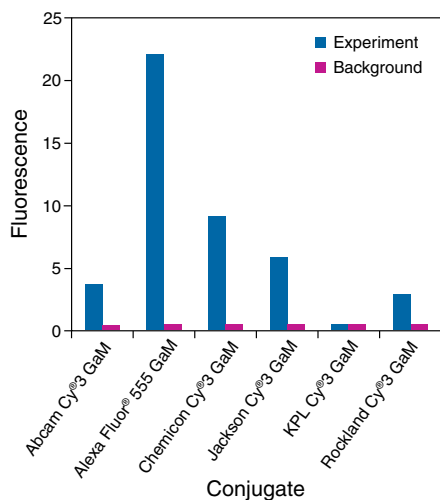
Figure 1—Emission spectra and spectral characteristics of the Alexa Fluor® dye series.



**Figure 2—A gallery of images highlighting detection with Alexa Fluor® conjugates.** A. Alexa Fluor® 488 rabbit anti-mouse IgG, Alexa Fluor® 594 goat anti-rabbit IgG, DAPI. Image contributed by Neville Cobbe, University of Edinburgh. B. pShooter vector pCMV/myc/mito/GFP, Alexa Fluor® 594 wheat germ agglutinin. C. Anti- $\alpha$ -tubulin antibody, Alexa Fluor® 350 goat anti-mouse IgG, Alexa Fluor® 594 phalloidin, Alexa Fluor® 488 wheat germ agglutinin. D. Anti-fibronectin antibody, Alexa Fluor® 488 goat anti-chicken IgG, Alexa Fluor® 594 wheat germ agglutinin, Alexa Fluor® 680 phalloidin, DAPI. E. Anti-golgin 97 antibody, Alexa Fluor® 647 goat anti-mouse IgG, SYTOX® Orange stain. F. Alexa Fluor® 488 Monoclonal Antibody Labeling Kit, Alexa Fluor® 594 goat anti-mouse IgG. Image contributed by Uffe Birk Jensen, University of Aarhus, Denmark.



**Figure 3**—Comparison of the relative fluorescence of goat anti-mouse IgG antibody conjugates prepared from the Alexa Fluor<sup>®</sup> 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 4**—Brightness comparison of Alexa Fluor<sup>®</sup> 555 goat anti-mouse IgG antibody and Cy<sup>®</sup>3 goat anti-mouse IgG antibody conjugates commercially available from several other companies. Human blood was blocked with normal goat serum and incubated with an anti-CD3 mouse monoclonal antibody; cells were washed, resuspended, and incubated with either the Alexa Fluor<sup>®</sup> 555 or Cy<sup>®</sup>3 goat anti-mouse IgG antibody at equal concentrations. Red blood cells were lysed and the samples were analyzed with a flow cytometer equipped with a 488 nm argon-ion laser and a 585 ± 21 nm bandpass emission filter.

- Brightness—Alexa Fluor<sup>®</sup> conjugates exhibit more intense fluorescence than other spectrally similar conjugates (Figures 3 and 4)
- Photostability—Alexa Fluor<sup>®</sup> conjugates are more photostable than most other fluorescent conjugates, allowing you to get the most signal from your sample (Figure 5)
- pH insensitivity—Alexa Fluor<sup>®</sup> dyes remain highly fluorescent over a broad pH range (Figure 6)
- Instrument compatibility—Alexa Fluor<sup>®</sup> conjugates have absorption spectra that are matched to the principal output wavelengths of common excitation sources, and can easily be swapped in for conventional fluorophores (Table 1)

### Find the conjugate you need

Invitrogen provides one of the largest selections of fluorescent secondary antibodies available anywhere (Table 2). Whether you use fluorescence microscopy, flow cytometry, microplate assays, or blotting techniques, our secondary detection conjugates can enhance your experiments with their bright fluorescent signals and low background. Alexa Fluor<sup>®</sup> conjugates exhibit superior brightness and photostability, outperforming most conventional fluorescent secondary reagents across the spectrum.

**Table 1**—Alexa Fluor<sup>®</sup> dye equivalents of commonly used fluorescent dyes

If you're using ...	Try this Alexa Fluor <sup>®</sup> dye
AMCA, coumarin	Alexa Fluor <sup>®</sup> 350
Cy <sup>®</sup> 2 dye, FITC (fluorescein)	Alexa Fluor <sup>®</sup> 488
Cy <sup>®</sup> 3 dye, TRITC (tetramethylrhodamine)	Alexa Fluor <sup>®</sup> 555
Rhodamine Red <sup>™</sup> dye	Alexa Fluor <sup>®</sup> 568
Texas Red <sup>®</sup> dye	Alexa Fluor <sup>®</sup> 594
Cy <sup>®</sup> 5 dye	Alexa Fluor <sup>®</sup> 647
Cy <sup>®</sup> 5.5 dye	Alexa Fluor <sup>®</sup> 680

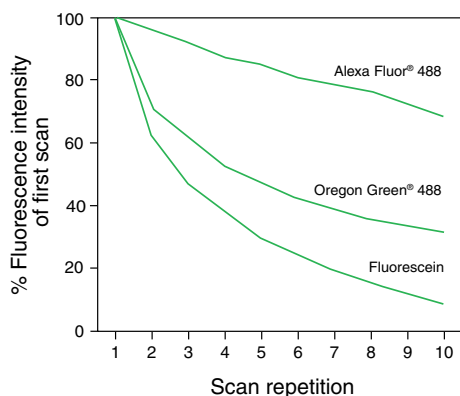
## Improve signal-to-noise ratio and reduce photobleaching

### Image-iT™ FX Signal Enhancer

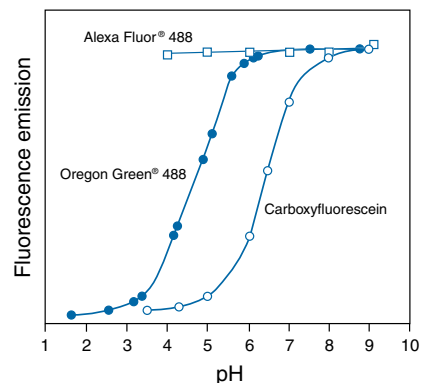
Image-iT™ FX Signal Enhancer dramatically improves the signal-to-noise ratio of immunolabeled cells and tissues, allowing you to clearly visualize targets that would normally be indistinguishable (Figure 7). Background staining typically seen with fluorescent dyes is largely eliminated when Image-iT™ FX Signal Enhancer is applied to fixed and permeabilized cells before staining.

### ProLong® Gold Antifade Reagent

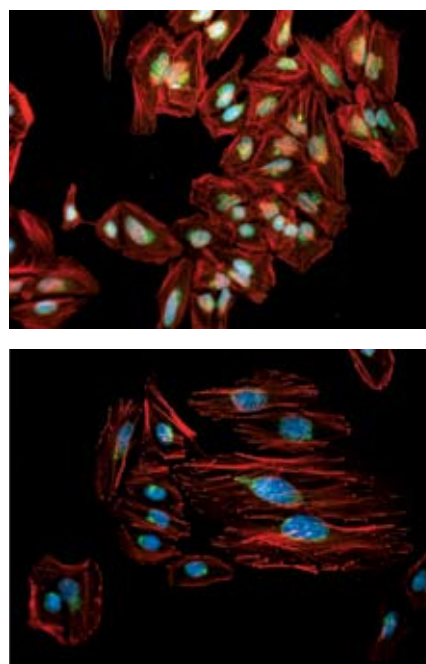
ProLong® Gold Antifade Reagent is an improved version of the very effective ProLong® Antifade Reagent (Figure 8, left panel). This new reagent is premixed and ready to use—just add a drop



**Figure 5—Photobleaching comparison of Alexa Fluor® 488, Oregon Green® 488, and fluorescein dyes.** EL4 cells were labeled with biotin-conjugated anti-CD44 antibody and detected by streptavidin conjugates of Alexa Fluor® 488, Oregon Green® 488, or fluorescein dyes. Labeled cells were fixed, washed, wet-mounted, then scanned 10 times successively on a laser-scanning cytometer (5 min per scan at 25 mW for the 488 nm spectral line of the argon-ion laser). Data are the mean fluorescence intensity (MFI) of each scan divided by the MFI of the first scan, expressed in percentages. Data contributed by Bill Telford, Experimental Transplantation and Immunology Branch, National Cancer Institute.



**Figure 6—Comparison of pH-dependent fluorescence of the Oregon Green® 488, carboxyfluorescein, and Alexa Fluor® 488 fluorophores.** Fluorescence intensities were measured for equal concentrations of the three dyes using excitation/emission at 490/520 nm.



**Figure 7—Increased label specificity and resolution provided by Image-iT™ FX Signal Enhancer.** Fixed and permeabilized bovine pulmonary artery endothelial cells were treated with Image-iT™ FX Signal Enhancer (bottom) or left untreated (top) and then labeled with tetramethylrhodamine streptavidin.



Table 2—Alexa Fluor® secondary antibody conjugates.

Antibody	Host	Alexa Fluor® 350	Alexa Fluor® 488	Alexa Fluor® 555	Alexa Fluor® 568	Alexa Fluor® 594
Anti-Chicken IgG	Goat		A11039	A21437	A11041	A11042
Anti-Goat IgG	Chicken		A21467			A21468
Anti-Goat IgG	Donkey	A21081	A11055	A21432	A11057	A11058
Anti-Goat IgG	Rabbit		A11078	A21431	A11079	A11080
Anti-Guinea Pig IgG (highly cross-adsorbed)	Goat		A11073	A21435	A11075	A11076
Anti-Human IgG	Goat		A11013	A21433	A21090	A11014
Anti-Mouse IgM (μ chain)	Goat	A31552	A21042	A21426	A21043	A21044
Anti-Mouse IgG	Chicken		A21200			A21201
Anti-Mouse IgG	Donkey		A21202	A31570		A21203
Anti-Mouse IgG	Goat	A11045	A11001	A21422	A11004	A11005
Anti-Mouse IgG (highly cross-adsorbed)	Goat	A21049	A11029	A21424	A11031	A11032
Anti-Mouse IgG1 (γ1)	Goat	A21120	A21121	A21127	A21124	A21125
Anti-Mouse IgG2a (γ2a)	Goat	A21130	A21131	A21137	A21134	A21135
Anti-Mouse IgG2b (γ2b)	Goat	A21140	A21141	A21147	A21144	A21145
Anti-Mouse IgG, F(ab') <sub>2</sub> fragments	Goat	A11068	A11017	A21425	A11019	A11020
Anti-Mouse IgG	Rabbit	A21062	A11059	A21427	A11061	A11062
Anti-Rabbit IgG	Chicken		A21441			A21442
Anti-Rabbit IgG	Donkey		A21206	A31572		A21207
Anti-Rabbit IgG	Goat	A11046	A11008	A21428	A11011	A11012
Anti-Rabbit IgG (highly cross-adsorbed)	Goat	A21068	A11034	A21429	A11036	A11037
Anti-Rabbit IgG, F(ab') <sub>2</sub> fragments	Goat	A11069	A11070	A21430	A21069	A11072
Anti-Rat IgG	Chicken		A21470			A21471
Anti-Rat IgG	Donkey		A21208			A21209
Anti-Rat IgG	Goat	A21093	A11006	A21434	A11077	A11007
Anti-Rat IgG	Rabbit		A21210			A21211
Anti-Sheep IgG	Donkey	A21097	A11015	A21436	A21099	A11016
Streptavidin	NA	S11249	S32354* S11223	S32355* S21381	S11226	S32356* S11227

\* 2 mg/ml in solution. NA = not applicable. Additional antibody and dye selections are also available. Please visit [probes.invitrogen.com](http://probes.invitrogen.com) to view our entire offering of secondary antibody conjugates.

Alexa Fluor® 633	Alexa Fluor® 635	Alexa Fluor® 647	Alexa Fluor® 680	Cross-adsorption
A21103		A21449		Not cross-adsorbed
		A21469		Human, mouse, and rabbit IgGs
A21082		A21447	A21084	Human, mouse, rat, and rabbit IgGs
A21086		A21446	A21088	Human and rat sera
A21105		A21450		Bovine, chicken, goat, hamster, human, mouse, rabbit, rat, and sheep sera
A21091		A21445		Bovine, mouse, and rabbit sera
A21046		A21238	A21048	Human IgA, IgG, serum, and paraproteins
		A21463		Human IgG
		A31571		Bovine, chicken, goat, guinea pig, hamster, horse, human, rabbit, rat, and sheep sera
	A31574	A21235	A21057	Human IgA and IgG
	A31575	A21236	A21058	Bovine, goat, human, rabbit, and rat IgGs; human serum
A21126		A21240	A31562	Mouse IgM, IgA, IgG2a, IgG2b, and IgG3; pooled sera and purified human paraproteins
A21136		A21241	A31563	Mouse IgM, IgA, IgG1, IgG2b, and IgG3; pooled sera and purified human paraproteins
A21146		A21242	A31564	Mouse IgM, IgA, IgG1, IgG2a, and IgG3; pooled sera and purified human paraproteins
A21053		A21237	A21059	Human IgG and serum
A21063		A21239	A21065	Human serum
		A21443		Human and mouse IgGs
		A31573		Bovine, chicken, goat, guinea pig, hamster, horse, human, mouse, rat, and sheep sera
	A31576	A21244	A21076	Human and mouse IgGs and sera, bovine serum
	A31577	A21245	A21109	Bovine, goat, human, mouse, and rat IgGs
A21072		A21246	A21077	Human paraproteins and serum, mouse plasmacytoma/hybridoma proteins and serum
		A21472		Human and rabbit IgGs
				Bovine, chicken, goat, guinea pig, hamster, horse, human, mouse, rabbit, and sheep sera
A21094		A21247	A21096	Mouse IgG and serum, human serum
				Human IgG
A21100		A21448	A21102	Human IgG and serum; bovine, mouse and rabbit sera
	S32364	S32357* S21374	S32358* S21378	NA

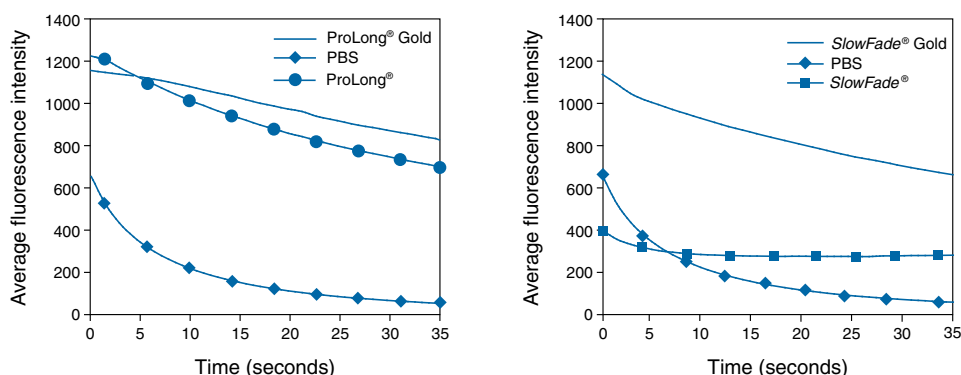


## Imaging Tools

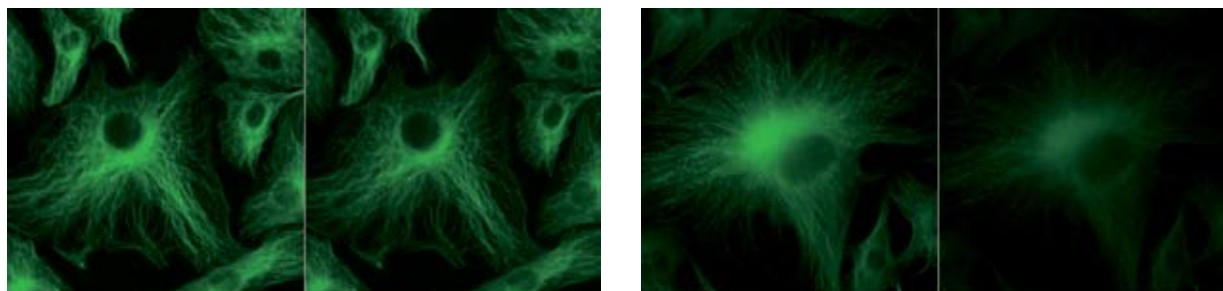
to your preparation and mount. ProLong® Gold reagent outperforms most other commercially available antifade reagents, and significantly reduces fluorophore photobleaching while causing little or no quenching of the fluorescence signal (Figure 9). Additionally, it is water miscible for spot-free images. This reagent offers excellent compatibility with a multitude of dyes and dye complexes, making it an especially valuable tool for multicolor applications. ProLong® Gold reagent cures within 24 hours, and the sample can be saved for months after mounting. ProLong® Gold Antifade Reagent is also available with DAPI nucleic acid stain to reduce extra labeling steps in your experiments.

## SlowFade® Gold Antifade Reagent

SlowFade® Gold Antifade Reagent (Figure 8, right panel) outperforms the original SlowFade® reagent, offering increased resistance to photobleaching for a wide range of fluorescent dyes. SlowFade® Gold reagent is provided as a premixed and ready-to-use solution. Unlike ProLong® Gold reagent, SlowFade® Gold reagent does not cure over time, so samples can be viewed immediately—simply tack the corners of the slide with hot wax or nail polish, then image. SlowFade® Gold reagent is intended for short-term use (3–4 weeks) only; samples mounted with it may degrade over time. SlowFade® Gold Antifade Reagent is also available with DAPI.



**Figure 8—Superior performance of ProLong® Gold and SlowFade® Gold Antifade Reagents.** Fluorescein-labeled microspheres were mounted with various antifade reagents and illuminated for 30 seconds using a 100 W Hg-arc lamp. Samples were imaged using a 40x/1.3 NA oil immersion lens and acquired using a 12-bit monochrome CCD camera. The same exposure settings were used for all images. Each data point plotted is the average fluorescence intensity from 20 microspheres.



**Figure 9—Enhanced resistance to photobleaching afforded by ProLong® Gold Antifade Reagent.** Fixed bovine pulmonary artery endothelial cells were labeled with anti- $\alpha$ -tubulin and visualized with fluorescein goat anti-mouse IgG. The samples were mounted in ProLong® Gold Antifade Reagent (left) or phosphate buffered saline (right). Images were acquired at 0 and 5 seconds, using a 40x/1.3 NA oil immersion objective with continuous illumination from a standard 100 W Hg-arc lamp.

## Get unsurpassed imaging results with imaging kits

The combination of bright, photostable Alexa Fluor® conjugated secondary antibodies, Image-iT™ FX Signal Enhancer, and ProLong® Gold Antifade Reagent enables you to create better images right from the start. To help you achieve these results, we have put these products together in different combinations designed to fit the specific requirements of your experiments.

### Image-iT™ FX Kits

The Image-iT™ FX Kits (Table 3) provide a complete starter package for the researcher setting up a new lab or training a new scientist. They combine the secondary detection reagents and supporting materials needed for optimal imaging of fixed cells and tissue sections: the most commonly used Alexa Fluor® dye secondary antibody and streptavidin conjugates; ProLong® Gold Antifade

Reagent to further reduce photobleaching; Image-iT™ FX Signal Enhancer to improve signal-to-noise ratio; and two CultureWell™ Chambered Coverglasses (see “Sample Chambers, slides, and coverslips”) for convenient sample processing.

### Alexa Fluor® SFX Kits

Alexa Fluor® SFX Kits (Table 3) provide a great way to try out different Alexa Fluor® dye conjugates. If you are primarily using Alexa Fluor® 488 conjugates but need an additional conjugate with a different emission color, then the smaller amount of antibody in these kits makes it more economical for you. Additionally, these kits are perfect for small-scale experiments. The kits include 200 µl of secondary antibody conjugate (2 mg/ml) and the Image-iT™ FX Signal Enhancer.

Table 3—Alexa Fluor® antibody imaging kits.

Kit	Fluorescent dye				
	Alexa Fluor® 350	Alexa Fluor® 488	Alexa Fluor® 555	Alexa Fluor® 594	Alexa Fluor® 647
SFX Kit (goat anti-mouse conjugate)	NA	A31619 A31620*	A31621 A31622*	A31623 A31624*	A31625 A31626*
SFX Kit (goat anti-rabbit conjugate)	NA	A31627 A31628*	A31629 A31630*	A31631 A31632*	A31633 A31634*
Image-iT™ FX Kit (goat anti-mouse IgG conjugate)	I37150	I37151	I37152	I37153	I37154
Image-iT™ FX Kit (goat anti-rabbit IgG conjugate)	I37155	I37156	I37157	I37158	I37159
Image-iT™ FX Kit (streptavidin conjugate)	I37160	I37161	I37162	I37163	I37164

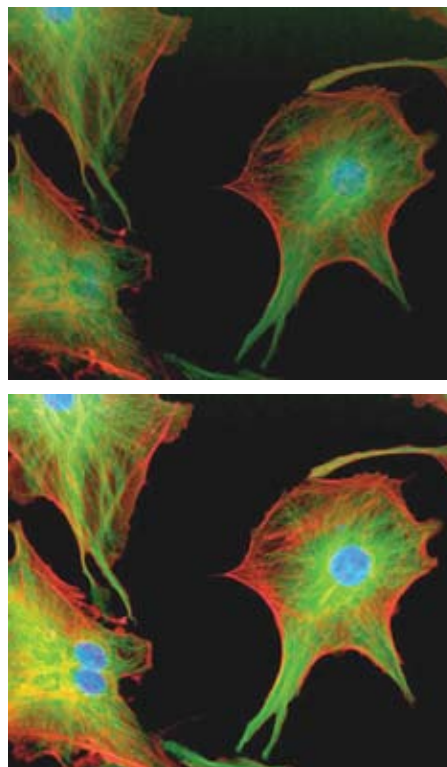
\*Highly cross adsorbed; see Table 2 for cross-adsorbed species. NA = not available in kit format. For current prices, please visit [probes.invitrogen.com](http://probes.invitrogen.com).



## Ensure exceptional optical performance

### Semrock BrightLine® filters

Semrock's BrightLine® high-performance optical filters—the brightest and most durable fluorescence filters—are now available from Invitrogen. Offered for every commonly used wavelength (Table 4), these excitation and emission filters deliver exceptional optical performance, including maximal brightness, superior contrast and sharpness (Figure 10), fast measurements, and durability. BrightLine® filters are made using Semrock's unique single-substrate construction, which avoids scattering, absorption, and reflection losses that occur with traditional multi-substrate laminated filters, which have adhesives in the optical path. When combined with Semrock's state-of-the-art ion-beam sputtering deposition technology, the result is the highest possible transmission of any fluorescence filters on the market. All BrightLine® filters withstand high optical intensities—they have been tested with xenon and halogen broadband light sources for prolonged periods with no noticeable degradation.



**Figure 10—Superior performance of BrightLine® filters.** Bovine pulmonary artery endothelial cells (FluoCells® prepared slide #2) imaged with filters from the leading competitor (top), and with a BrightLine® multiband DAPI/FITC/Texas Red® filter set (bottom). Images were captured under identical conditions on an Olympus BX41 microscope using a 40x objective.

**Table 4—Semrock BrightLine® Filter Sets.**

Filter set	Filter set configuration					
	Unmounted	Unmounted, zero pixel shift	Nikon Quadfluor	Nikon TE2000	Olympus UMF2	Zeiss Axio AF
Alexa Fluor® 350 Filter Set (Semrock BrightLine®)	A37506	A37507	A37508	A37510	A37512	A37514
Alexa Fluor® 488 Filter Set (Semrock BrightLine®)	A37516	A37517	A37518	A37520	A37522	A37524
Alexa Fluor® 555 Filter Set (Semrock BrightLine®)	A37526	A37527	A37528	A37530	A37532	A37534
Alexa Fluor® 594 Filter Set (Semrock BrightLine®)	A37536	A37537	A37538	A37540	A37542	A37544
Alexa Fluor® 647 Filter Set (Semrock BrightLine®)	A37546	A37547	A37548	A37550	A37552	A37554
Alexa Fluor® 350/488/594 Filter Set (Semrock BrightLine®)	A37556	NA	A37557	A37558	A37559	A37560
Fura-2 Filter Set (Semrock BrightLine®)	F37561	F37562	NA	NA	NA	NA

Zero pixel shift single-color mounted filter sets are also available. For more information, visit our website at [probes.invitrogen.com](http://probes.invitrogen.com). NA=not available.

## Keep your microscopes calibrated and aligned

Fluorescent microspheres designed as reference standards for various microscopy applications (Table 5) will help you obtain the best images from your fluorescent samples. These standards include:

- FocalCheck™ fluorescent microspheres for assessing alignment and stability (Figure 11, Table 6)
- FocalCheck™ fluorescence microscope test slides—each containing 10 distinct sample areas—for calibrating fluorescence microscope systems and evaluating system and filter performance (Table 7)

Table 7—FocalCheck™ fluorescence microscope test slides.

FocalCheck™ Test Slide	Recommended use	Cat. no.
Slide #1	Routine checking and calibration of confocal and widefield fluorescence microscopes	F36909
Slide #2	Evaluation of performance of spectral imaging systems, discrimination of closely overlapping spectra	F36913
Slide #3	Basic evaluation of filter performance, general practice for fluorescence microscopy and digital imaging	F36914

\* The approximate fluorescence excitation and emission maxima, in nm, of the ring and core stains are: blue, 365/430; green, 505/515; orange, 560/580; and dark red, 660/680. For current prices, please visit our website at [probes.invitrogen.com](http://probes.invitrogen.com).

Table 5—Fluorescent microspheres for the calibration of confocal and widefield epifluorescence.

	Image registration; X,Y alignment	Point spread function (PSF) measurement	Z-step calibration	Relative intensity measurement	Spectral unmixing	Color range *	Premounted on slides
FocalCheck™	●		●		● †	B/G/O/R/NIR ‡	●
MultiSpeck™	●					B/G/R	
TetraSpeck™	●	● (0.2 μm beads)				B/G/O/NIR	●
PS-Speck™		●				B/G/O/NIR	
LinearFlow™				●		B/G/O/R/NIR	

\* Nominal fluorescent colors are: B = blue, G = green, R = red, O = orange, and NIR = near infrared. See individual products for excitation and emission maxima. Product names for microspheres fluorescing in the near infrared range are "deep red" or "dark red." † FocalCheck™ beads useful for spectral unmixing are the DoubleGreen, DoubleOrange, DoubleRed, and DoubleFarRed microspheres. ‡ The FocalCheck™ product line contains several product configurations, including beads that emit two distinct fluorescent colors. See individual FocalCheck™ products for details on specific color combinations.

Table 6—FocalCheck™ fluorescent microsphere suspensions.

Ring stain *	Core stain	Bead size (μm)	Cat. no.
Blue, orange	None	15	F7234
Green, dark red	None	15	F7240
Green, orange, dark red	None	15	F7235
Green, orange, dark red	None	6	F14806
Green	Blue	15	F7237
Green	Blue	6	F14808
Green	Dark red	15	F7238
Orange	Blue	15	F7236
Dark red	Green	15	F7239
Dark red	Green	6	F14807

\* The approximate fluorescence excitation and emission maxima, in nm, of the ring and core stains are: blue, 365/430; green, 505/515; orange, 560/580; and dark red, 660/680. For current prices, please visit our website at [probes.invitrogen.com](http://probes.invitrogen.com).

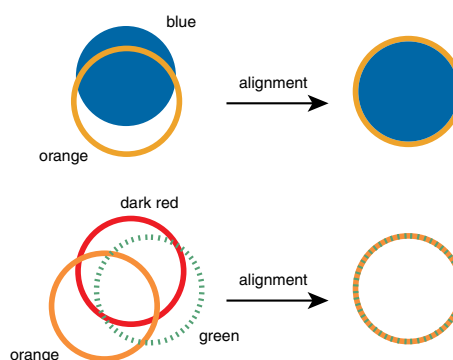


Figure 11—Optical alignment using FocalCheck™ microspheres. The microspheres with orange ring stain and blue throughout allow UV/visible wavelength alignment in three dimensions upon aligning the orange ring with the blue disk (top row). Focal alignment is also possible simultaneously in three colors by aligning the three rings of the FocalCheck™ microspheres containing fluorescent green, orange, and dark red ring stains (bottom row).



- MultiSpeck™ and TetraSpeck™ fluorescent microspheres for examining chromatic registration, resolution, and instrument sensitivity. Each microsphere is stained with three (Multi-Speck™) or four (TetraSpeck™) dyes (Table 8)
- PS-Speck™ Microscope Point-Source Kit for calibrating microscope optics. The kit includes separate bead suspensions of four fluorescent colors: blue (365/415 nm), green (505/515 nm), orange (540/560 nm), and deep red (633/660 nm)
- LinearFlow™ fluorescent microspheres for intensity calibration (Figure 12, Table 9)

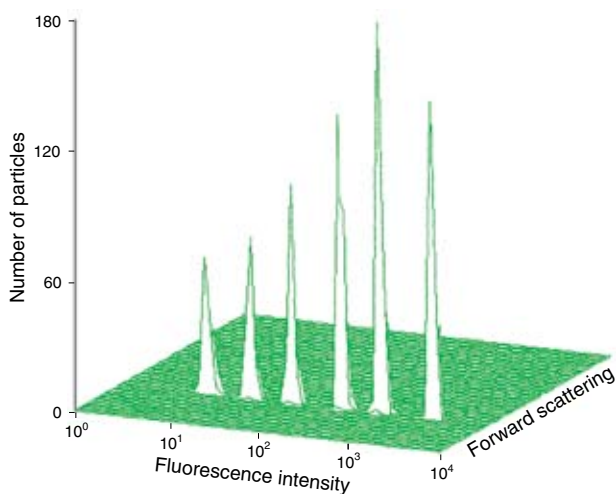


Figure 12—Flow cytometric analysis of LinearFlow™ Green microspheres with nominal relative fluorescence intensities of 100%, 30%, 10%, 3%, 1%, and 0.3%. For each lot, actual relative intensities are determined by flow cytometry and printed on the product labels.

### Image Intensity Calibration Kits

LinearFlow™ Intensity Calibration Kits (Table 9) contain microsphere suspensions for use as fluorescence intensity reference standards. These beads can be used to generate calibration curves and evaluate sample brightness:

- Each kit includes separate bead suspensions of 100%, 30%, 10%, 3%, 1%, 0.3%, and 0% relative fluorescence intensity (Figure 12)
- Kits are available in five fluorescent colors: blue (350/440 nm), green (505/515 nm), orange (540/560 nm), red (580/605), and deep red (633/660 nm)
- Beads are available in 2.5 or 6 μm diameter

Table 8—MultiSpeck™ and TetraSpeck™ fluorescent microspheres.

Microsphere	Nominal bead diameter (μm)	Cat. no.
Blue, green, red fluorescence *		
MultiSpeck™	4	M7901 †
Blue, green, orange, dark red fluorescence *		
TetraSpeck™	0.1	T7279
TetraSpeck™	0.2	T7280
TetraSpeck™	0.5	T7281
TetraSpeck™	1.0	T7282
TetraSpeck™	4.0	T7283

\* Corresponding excitation and emission peaks are 365/430 nm (blue), 505/515 nm (green), 560/580 nm (orange), 580/600 nm (red), and 660/680 nm (dark red). † Contains a suspension of MultiSpeck™ beads and a mixed suspension of beads singly labeled with each of the three dyes.

Table 9—LinearFlow™ Intensity Calibration Kits.

LinearFlow™ Kit	Number of intensity levels	Nominal bead diameter (µm)	Ex/Em*	Cat. no.
LinearFlow™ Blue	4, plus nonfluorescent beads	2.5	350/440	L14812
		6		L14813
LinearFlow™ Green	4, plus nonfluorescent beads	2.5	505/515	L14821
		6		L14822
LinearFlow™ Orange	4, plus nonfluorescent beads	2.5	540/560	L14814
		6		L14815
LinearFlow™ Carmine	4, plus nonfluorescent beads	2.5	580/605	L14816
		6		L14817
LinearFlow™ Deep Red	4, plus nonfluorescent beads	2.5	633/660	L14818
		6		L14819

\* Approximate fluorescence excitation (Ex) and emission (Em) maxima for the stained microspheres, in nm.

## Sample chambers, slides and coverslips

In collaboration with Grace Bio-Labs, we offer a collection of accessories for imaging and microscopy. These accessories make slide preparation easy, facilitate sample perfusion, and simplify sample manipulation during *in situ* hybridization and other procedures that involve multiple wash steps. A sample of these accessories are described here—please see our product handbook for more complete listings and product details.

### CultureWell™ Cell Culture Systems

CultureWell™ Cell Culture Systems (Figure 13) provide an integrated set of tools for preparing cultured cells for staining and imaging. Each system uses medical-grade silicone gaskets preassembled with standard optical-quality coverslips into convenient inserts that fit into matching cell culture plates.

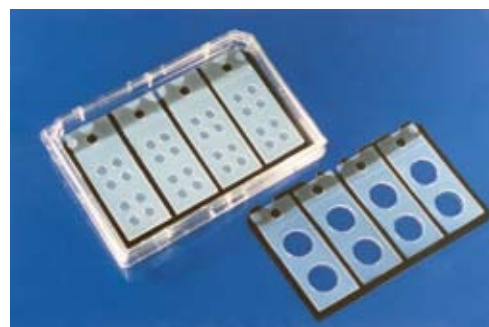


Figure 13—CultureWell™ cell culture system.



## Imaging Tools

### CultureWell™ Chambered Coverglasses

CultureWell™ Chambered Coverglasses (Figure 14) for cell culture are ideal for cell culture and fluorescence imaging applications. The chambered coverglass—provided sterile and ready to use—contains 16 wells that can each hold up to 250  $\mu$ l, allowing cells to be cultured in a number of different conditions on a single slide. The coverslip can be removed from the chambers and mounted as usual for imaging experiments.



Figure 14—CultureWell™ chambered removable coverglass.

### CoverWell™ Imaging Chamber Gaskets

CoverWell™ Imaging Chamber Gaskets (Figure 15) incorporate a thin, optically clear plastic cover, making them ideal for light, epifluorescence, and confocal laser-scanning microscopy. By simply pressing an imaging chamber gasket to a microscope slide or coverslip, a sealed chamber is formed to contain mounting medium. The watertight chamber supports and stabilizes thick and free-floating specimens, permitting resolution of fine internal structures and analysis of markers without the compression or movement artifacts that affect observations made using an ordinary coverslip.

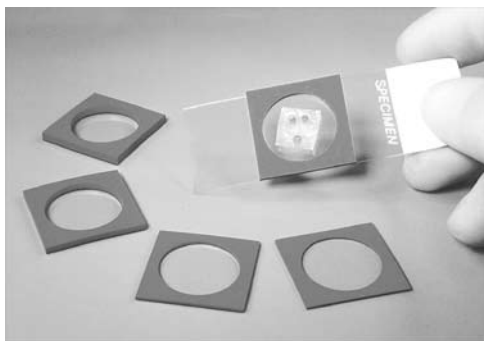


Figure 15—CoverWell™ imaging chamber gaskets.

### ONCYTE® MultiWells

ONCYTE® MultiWells (Figure 16) consist of a two-piece set that includes a slide printed with nitrocellulose circles and a matching removable gasket to enclose and isolate each sample. The nitrocellulose coating on the slide is specially formulated for fluorescence imaging. This ultrathin microporous coating ensures uniform binding of tissue prints, cells, or macromolecules and becomes transparent in a variety of mounting media. The matching press-to-seal silicone gaskets adhere easily to the surface of the slide to isolate specimens and reagents and prevent cross-contamination.

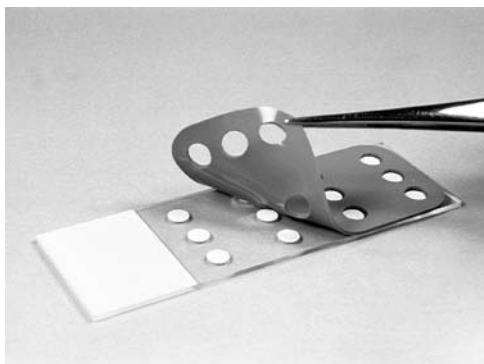


Figure 16—ONCYTE® MultiWells.

## Ordering Information

Product	Quantity	Cat. no.
<b>Imaging reagents</b>		
Image-iT™ FX Signal Enhancer	10 ml	I36933
ProLong® Gold Antifade Reagent	10 ml	P36930
ProLong® Gold Antifade Reagent	5 × 2 ml	P36934
ProLong® Gold Antifade Reagent with DAPI	10 ml	P36931
ProLong® Gold Antifade Reagent with DAPI	5 × 2 ml	P36935
SlowFade® Gold Antifade Reagent	10 ml	S36936
SlowFade® Gold Antifade Reagent	5 × 2 ml	S36937
SlowFade® Gold Antifade Reagent with DAPI	10 ml	S36938
SlowFade® Gold Antifade Reagent with DAPI	5 × 2 ml	S36939
<b>Imaging accessories</b>		
CultureWell™ Multiwell Cell Culture System	set of 2	C24767
CultureWell™ Chambered Coverglass for cell culture, sixteen wells per coverglass	set of 2	C37005
ONCYTE® MultiWells, 12 wells, 5 mm diameter, with slide and matching gasket	set of 20	O24750
For a complete listing of these products, please visit our web site <a href="http://probes.invitrogen.com">probes.invitrogen.com</a> .		

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invitrogen detection technologies

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