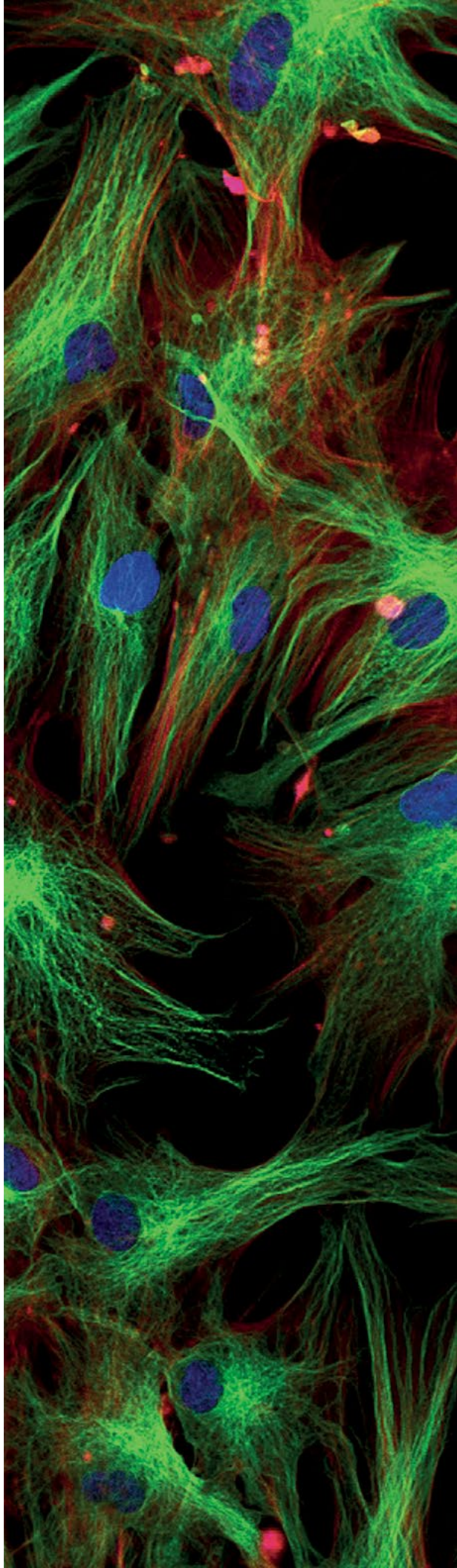
A fluorescence microscopy image showing a dense network of cells. The cytoplasm and some structures are stained green, the nuclei are stained blue, and other structures are stained red. The background is black.

# Fluorescent Dyes and Probes

Product Listing | Edition 2

biotechne® | TOCRIS





## Fluorescent Dyes and Probes

Bio-Techne, through the Tocris brand, offers a wide range of gold standard fluorescent dyes, as well as exclusive and spectrally enhanced dyes, designed and manufactured in-house. These include probes conjugated to the next generation Janelia Fluor® dyes, known for their brightness, superior photostability and applications in live cells.

We also provide fluorescent probes covering over 20 targets, aptamer-based RNA imaging reagents, enhanced Tyramide Signal Amplification (TSA) reagents and kits, and much more, to facilitate the visualization of sub-cellular components in live and fixed cells.

For up-to-date product listings, visit [tocris.com/fluorescence-imaging](https://tocris.com/fluorescence-imaging)

This brochure lists fluorescence imaging reagents conveniently organized by type for easy selection. Feature boxes throughout this listing identify the main principles of imaging technology and the application of corresponding product ranges. You will also find a flow cytometry table that groups dyes according to the most popular laser lines.

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Fluorescent Dyes

Fluorescent dyes, or fluorophores, enable researchers to identify, probe and visualize specific biological molecules using technologies including fluorescence microscopy and flow cytometry. Dyes can be classified according to some key features known as ‘photophysical’ properties, which include the maximum absorption and emission wavelengths ( $\lambda_{\text{abs}}$ / $\lambda_{\text{em}}$ ), and brightness, which is equal to extinction coefficient ( $\epsilon$ ) multiplied by quantum yield ( $\phi$ ). The Tocris range includes

the Janelia Fluor® Dyes, which are bright, highly photostable and cell-permeable, making them ideal for confocal fluorescent imaging and super-resolution microscopy (SRM) techniques in live and fixed cells. Also included in our portfolio are novel fluorophores in the near-IR range, for deep tissue and *in vivo* imaging work, together with a comprehensive palette of well known, gold standard dyes for all your imaging requirements.

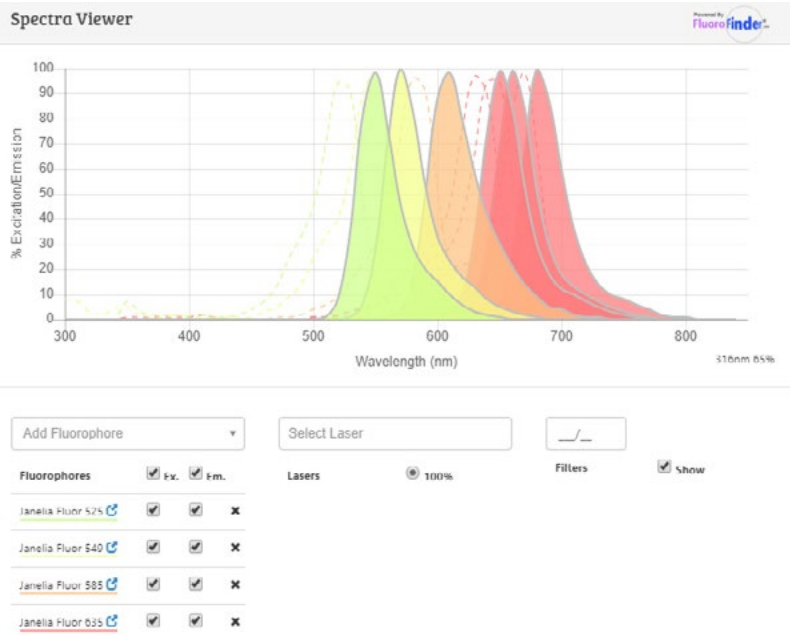
Emission Color	Product Name	Reactive Group	Cat. No.	$\lambda$ Abs (nm)	$\lambda$ Em (nm)
Blue	3-Azido-7-hydroxycoumarin	Azide	7664	404	477
	Ocean Blue	NHS ester	6489	405	455
Green	BDY FL	NHS ester	5465	502	510
	FITC	Isothiocyanate	5440	495	525
Yellow	Janelia Fluor® 525	NHS ester	6296	525	549
	HM Janelia Fluor® 526	NHS ester	7312	526	550
	Janelia Fluor® 526	NHS ester	7316	526	550
	PA Janelia Fluor® 549	NHS ester	6149	553	573
	Janelia Fluor® 549	Maleimide	6500	549	571
		Tetrazine	6502	549	571
		NHS ester	6147	549	571
		Free acid	6503	549	571
Orange/Red	Janelia Fluor® 585	NHS ester	6418	585	609
	Janelia Fluor® 635	NHS ester	6419	635	652
	PA Janelia Fluor® 646	NHS ester	6150	651	665
	Janelia Fluor® 646	Maleimide	6590	646	664
		Free acid	6993	646	664
		Azide	7088	646	664
		Tetrazine	7279	646	664
		NHS ester	6148	646	664
	5-TAMRA	NHS ester	7665	546	580
	Cyanine 5	NHS ester	5436	649	666

Emission Color	Product Name	Reactive Group	Cat. No.	$\lambda$ Abs (nm)	$\lambda$ Em (nm)
Far Red	BDY 650-X	NHS ester	5469	646	660
	Janelia Fluor® 669	NHS ester	6420	669	682
Near-IR	FNIR-Tag	NHS ester	7373	765	788
	Indocyanine green	-	7510	787	815
	NIR Dye s775z	NHS ester	7626	775	795

Spectra Viewer - Plan Your Experiments

Use our spectra viewer to interactively plan your experiments, assessing multiplexing options. View the excitation and emission spectra for our fluorescent dye range and other commonly used dyes.

Visit [tocris.com/resources/spectral-viewer](https://www.tocris.com/resources/spectral-viewer)



Novus Custom Conjugation

For pre-conjugated options, we also offer Janelia Fluor® conjugated antibodies and custom conjugation services through our sister brand, Novus Biologicals.

Visit [novusbio.com/products/janelia-florr](https://novusbio.com/products/janelia-florr)

BODIPY® is a registered trademark of Molecular Probes, Inc, a Thermo Fisher Scientific Company.



Next Generation Janelia Fluor® Dyes

Developed by Professor Luke Lavis and his team at the Janelia Research Campus, Janelia Fluor® (JF) dyes provide scientists with an exceptional palette of bright, photostable fluorophores for a broad range of applications, including super-resolution microscopy. The Janelia Fluor® range includes products with different specific and useful properties, such as: fluorogenicity; spontaneous blinking (for facile single-molecule localization microscopy (SMLM)); and photoactivation.

The full range of Janelia Fluor® dyes are available from Tocris with a selection of reactive groups for conjugation to biomolecules. Custom services are available if you need a modification not currently offered.

Conjugation protocols are available at [tocris.com/resources/protocols](https://www.tocris.com/resources/protocols)

We also offer Janelia Fluor® conjugated antibodies and custom conjugation services through our sister Bio-Techne brand Novus Biologicals. Visit [novusbio.com/products/janelia-fluor](https://www.novusbio.com/products/janelia-fluor)

Janelia Fluor® Dye Key Features and Applications

- Exceptionally bright
- Cell permeable
- Highly photostable
- Especially well-suited to live-cell imaging
- Supplied with a choice of reactive groups for simple biomolecule conjugation
- Can be converted to relevant substrate for use in self-labeling tag systems, e.g. HaloTag® and SNAP-tag®
- Suitable for use in confocal microscopy, IHC, ICC and flow cytometry
- Ideal for super-resolution techniques including STED and dSTORM
- Photoactivatable Janelia Fluor® dyes compatible with PALM microscopy

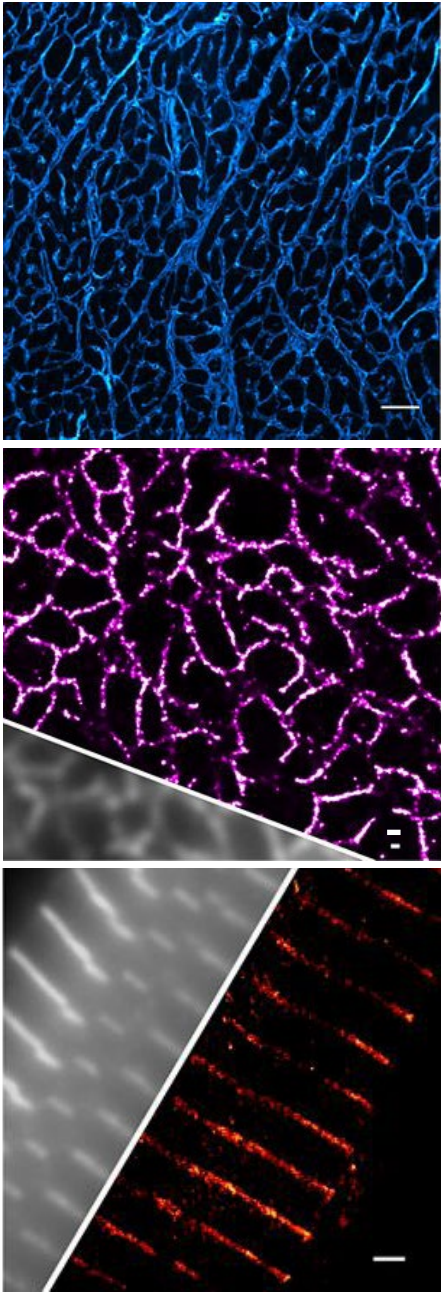
What Researchers are Saying about Janelia Fluor® Conjugates

**“We have been using the JF secondary antibodies and like them a lot for IHC. As advertised, they seem to be at least a little brighter and more photostable than the Alexa 647 and Cy3 dyes we had been using. We will probably switch over to these completely going forward.”**

- Steve Stowers, PhD, Assistant Professor, Montana State University

**“It’s a great antibody and great fluorophore by its signal sharpness and photostability.”**

- Anonymous



**Application of Janelia Fluor® Dyes in Cardiac Tissue:** Top – Widefield fluorescence image displaying the distribution of collagen VI in the interstitial space between muscle cells. Labeled with primary ab against collagen VI and secondary ab conjugated to JF549 (#6147). Scale: 50 µm. Middle – An adult pig heart tissue section, 10 µm thick, labeled with ab against SERCA2ATPase, exhibits the intricate structure of the sarcoplasmic reticulum. A super-resolution image (top), obtained by exploiting spontaneous photo-switching of JF549 (dSTORM), shows superior detail to the diffraction limited widefield image (bottom). Scale: 200 nm. Above – Rat cardiomyocyte stained against α-actinin, displaying its periodic structure localized at the ends of sarcomeres (Z-discs). Widefield fluorescence of JF549 (left), prior to illuminating with a 561 nm laser to induce photoswitching and produce a dSTORM super-resolution image (right). Scale: 1 µm. All images kindly provided by Prof. Christian Soeller, University of Exeter; acquired by Alex Clowsley and Anna Meletioui.

Flow Cytometry

Flow Cytometry is a laser-based technique that is used to count, sort, and characterize cells or particles in a single cell suspension. Traditional applications involve immunophenotyping of cells prepared from culture or tissue using fluorescent-dye conjugated antibodies that recognize specific cell surface or intracellular antigens.

Flow Cytometry Fluorophores for Common Laser (λ - nm)				
355 UV	405 Violet	488 Blue	561 Green-Yellow	640 Red
DAPI (5748)	DAPI (5748)	7-AAD (7121)	FITC (5440)	Cyanine 5, SE (5436)
Hoechst 33342 (5117)	Ocean Blue, SE (6489)	Calcein AM (5119)	Janelia Fluor® 549 <ul style="list-style-type: none"><li>• SE (6147)</li><li>• Tetrazine (6502)</li><li>• Free acid (6503)</li><li>• Maleimide (6500)</li></ul>	Janelia Fluor® 646 <ul style="list-style-type: none"><li>• SE (6148)</li><li>• Azide (7088)</li><li>• Free acid (6993)</li><li>• Maleimide (6590)</li></ul>
		H2DCFDA (5935)		
		MitoMark Green I (6444)		
		Propidium iodide (5135)		MitoBrilliant™ 646 (7700)
			MitoBrilliant™ Live 549 (7693)	MitoBrilliant™ Live 646 (7417)

Flow Cytometry Panel Builder Tool

Use our sister brand Novus’ flow cytometry panel builder tool to design your experiment; it enables you to find validated antibodies that work with specific cytometers. Your panel can be exported and saved for reference. Advanced features of this tool include a spectra viewer, spillover popups and antigen density selector.

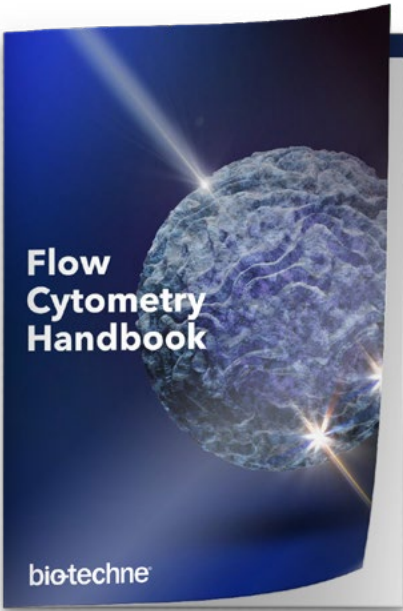
Visit [novusbio.com/flow-panel-builder](https://www.novusbio.com/flow-panel-builder)

Flow Cytometry Handbook

The Bio-Techne Flow Cytometry Handbook is the must have resource for researchers using flow cytometry.

Download now to see the step-by-step protocols and troubleshooting tips.

Visit [info.bio-techne.com/flow-cytometry-handbook](https://info.bio-techne.com/flow-cytometry-handbook)



Fluorescent Probes and Stains

Fluorescent probes target specific cellular and sub-cellular components, or they may selectively target a single biomolecule. Alternatively they might provide a functional read-out, such as live versus dead cell staining. They enable researchers to detect particular components of complex biomolecular assemblies,

such as microtubules, with high sensitivity and selectivity allowing the exploration of cell structure and function. Tocris offers a wide selection of fluorescent probes, covering organelle probes and cell viability stains.

	Cat.No.	Product Name	Description
Calcium Signaling Probes and Ca <sup>2+</sup> Indicator Dyes	6255	Fluo-4 AM	Cell-permeable, fluorescent Ca <sup>2+</sup> indicator
	2220	FURA-2AM	Fluorescent Ca <sup>2+</sup> indicator
	6704	Indo 1AM	Fluorescent Ca <sup>2+</sup> indicator
	3953	<i>cis</i> -Ned 19	Fluorescently labels NAADP receptors in intact cells; inhibits Ca <sup>2+</sup> release
	3954	<i>trans</i> -Ned 19	Fluorescently labels NAADP receptors in intact cells; inhibits Ca <sup>2+</sup> release
	6256	OG 488 BAPTA-1 AM	Cell-permeable, fluorescent Ca <sup>2+</sup> indicator
Cell Viability	5092	Acridine Orange	Emits green fluorescence when bound to double-stranded DNA and red fluorescence when bound to RNA or single-stranded DNA; used in cell cycle and apoptosis studies
	7121	7-Aminoactinomycin D	Detects apoptotic cells
	5119	Calcein AM	Cell permeable compound; hydrolyzed to become fluorescent in living cells
	5117	Hoechst 33342	Used as indicator of apoptosis; fluorescent dye for labeling DNA
	5135	Propidium Iodide	Red-fluorescent DNA stain; membrane impermeant to live cells. PI staining differentiates live and dead cells

Featured Calcium Signaling Probes and Ca<sup>2+</sup> Indicator Dye – FURA-2AM (Cat. No. 2220)

FURA-2AM is a fluorescent Ca<sup>2+</sup> indicator. It is selective for Ca<sup>2+</sup> over other divalent cations: Mg<sup>2+</sup>, Zn<sup>2+</sup>, Fe<sup>2+</sup> and Mn<sup>2+</sup>. FURA-2AM is used to determine intracellular Ca<sup>2+</sup> concentration.

FURA-2AM can be easily solubilized in F 127 (Cat. No. 6523). To see how our customers have used FURA-2AM view product citations at [tocris.com/products/fura-2am\\_2220#product-citations](https://www.tocris.com/products/fura-2am_2220#product-citations)

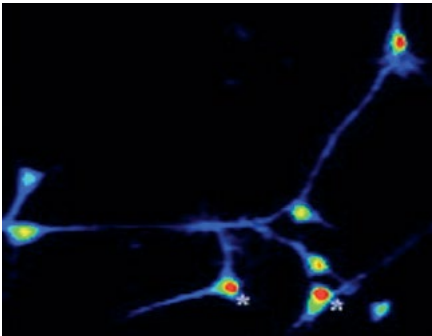
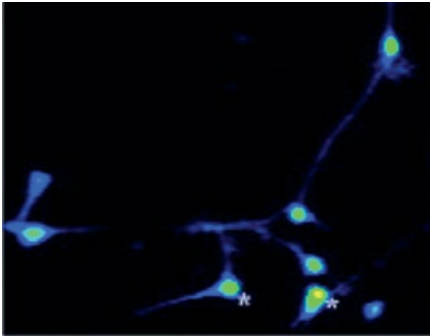
See what our customers think:

FURA-2AM ★★★★★ Reviews

Detects cytosolic calcium influx changes well

Used in live cell calcium imaging assay to study glutamate receptor signaling in neurons. Used at a concentration of 5 micromolar (along with pluronic). Images show changes in fura-2 fluorescence pre (see figure -top) and post (bottom) glutamate stimulation.

See more customer reviews for FURA-2AM at [fura-2a-reviews](#)



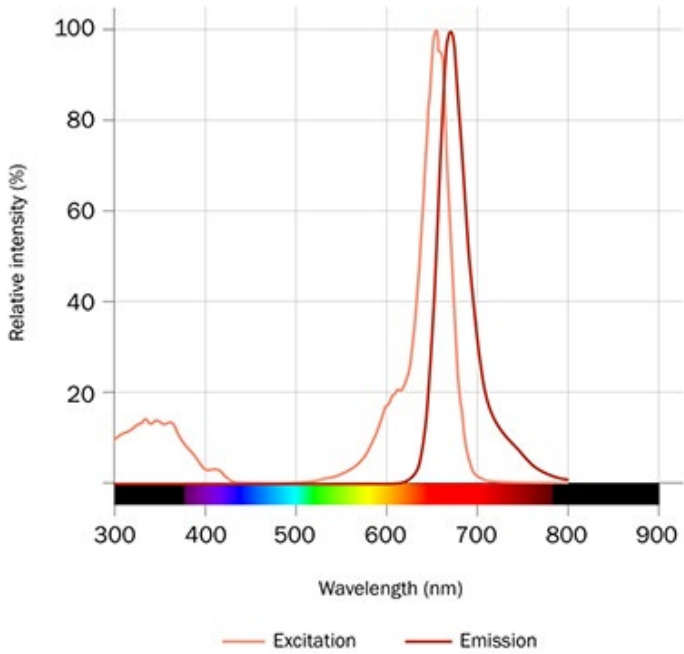
	Cat.No.	Product Name	Description
Enzyme Probes and Enzyme Substrates	7483	BDY FL VH032	High-affinity VHL fluorescent probe
	6882	DiFMUP	Fluorescent phosphatase substrate
	6204	7-Ethoxyresorufin	Fluorometric CYP450 substrate
	7287	FAM-DEALA-Hyp-YIPD	Fluorescent HIF-1α peptide
	7452	FAM-DEALAHypYIPMDDDFQLRSF	Fluorescent HIF-1α peptide
	7627	FITC-labelled Keap1-Nrf2 probe	Fluorescent Keap1-Nrf2 peptide
	6574	Fluorescein-NAD+	Fluorescent NAD+; substrate for ADP-ribosylation for use in PARP assays
	6461	PARPi-FL	Potent fluorescent PARP inhibitor; cell permeable
Fluorescent Actin Probes	4816	Pentoxeresorufin	Fluorometric cytochrome P450 substrate
	7288	Thalidomide-Cyanine 5	Fluorescent cereblon probe, λabsλem 650/665 nm
	5782	Phalloidin-FITC	Green fluorescent cytoskeleton stain
Fluorescent Amyloid β Probes	7201	Phalloidin-Janelia Fluor® 646	Red fluorescent F-actin probe
	5783	Phalloidin-TRITC	Red-orange fluorescent cytoskeleton stain
	5167	Congo Red	Amyloid fibril binding dye; also a VGlut inhibitor
	4803	CRANAD 2	Near-infrared probe that detects Aβ40 aggregates
	3144	K 114	Amyloid fibril-specific fluorescent dye
Fluorescent Cell Indicators and Sensors	4920	Methoxy-X04	Fluorescent amyloid β detector; brain penetrant
	7122	Thioflavin T	Fluorescent amyloid stain, binds to the stacked β sheets of amyloid fibrils
	5119	Calcein AM	Cell permeable compound; hydrolyzed to become fluorescent in living cells
	7324	Di 4 ANEPPS	Voltage-sensitive probe
	6594	GSHtracer	Ratiometric glutathione (GSH) probe
	5935	H2DCFDA	Fluorescent ROS indicator; cell permeable
	5085	L 012 sodium salt	Chemiluminescent ROS and RNS indicator
	7641	Mito-HE	Fluorescent mitochondrial superoxide indicator in live cells
	4428	MitoPY1	Fluorescent mitochondrial hydrogen peroxide indicator
	6065	2-NBDG	Fluorescent glucose uptake indicator
	4944	Peroxy Orange 1	Fluorescent hydrogen peroxide indicator
	6182	Pimonidazole	Hypoxia detection reagent
	4943	Sulfidefluor 7 AM	H2S fluorescent probe

	Cat.No.	Product Name	Description
Fluorescent Cholesterol Probes	6250	Filipin III	Fluorescent cholesterol stain; also polyene antibiotic
Fluorescent DNA Stains	5092	Acridine Orange	Nucleic acid binding dye
	7121	7-Aminoactinomycin D	Fluorescent DNA stain
	5748	DAPI	Fluorescent DNA stain
	5117	Hoechst 33342	Fluorescent dye for labeling DNA
	7313	Hoechst Janelia Fluor® 526	Fluorogenic, green-emitting DNA probe
	6804	Hoechst Janelia Fluor® 646	Fluorogenic, red-emitting DNA probe
	7246	Nucleic Acid Dye Green I	High affinity double-stranded DNA (dsDNA) probe; used in RT-LAMP and qPCR
	5135	Propidium Iodide	Red-fluorescent DNA stain; membrane impermeant to live cells. PI staining differentiates live and dead cells
Fluorescent Integrin Probes	6996	BOP-JF549	Yellow fluorescent dual $\alpha_v\beta_1/\alpha_4\beta_1$ integrin inhibitor; fluorogenic
	6997	BOP-JF646	Red fluorescent dual $\alpha_v\beta_1/\alpha_4\beta_1$ integrin inhibitor; fluorogenic
	4577	LDV FITC	Fluorescent ligand for $\alpha_4\beta_1$ (VLA-4)
	6048	R-BC154	High affinity fluorescent $\alpha_4\beta_1/\alpha_v\beta_1$ inhibitor; mobilizes HSCs

Featured Fluorescent DNA Stain  
– Hoechst Janelia Fluor® 646  
(Cat. No. 6804)

Hoechst Janelia Fluor® 646 is a fluorogenic red-emitting DNA probe; it preferentially stains and binds minor grooves of AT-rich regions. It can be combined with fluorogenic green-emitting DNA probe Hoechst Janelia Fluor® 526 for multiplexing experiments. This dye can also be combined with Hoechst Janelia Fluor® 526 to perform dual-color stimulated emission depletion microscopy (STED) using the same depletion laser ( $\lambda_{\text{dep}}$  = 775 nm). Sub-diffraction spatial resolution STED imaging can be achieved using Hoechst Janelia Fluor® 646 in *E. coli* (~100 nM - 1  $\mu$ M). This dye is a desirable alternative to large oligonucleotide-conjugated antibodies for PAINT (points accumulation for imaging in nanoscale topography) experiments, particularly for bacterial studies. The compound fluoresces only once bound to DNA, i.e. it is fluorogenic, enabling hassle-free no-wash experiments. It is suitable for multicolor microscopy experiments and for use in live-cell imaging.

Optical Data for Hoechst Janelia Fluor® 646



	Cat.No.	Product Name	Description
Fluorescent Lipid Probes and Cell Membrane Stains	7273	C-Laurdan	Two-photon polarity-sensitive lipid membrane probe
	5702	DiD perchlorate	Lipophilic fluorescent dye; shown to stain HSCs
	6250	Filipin III	Binds sterols in fungal membranes; fluorescent cholesterol stain; also polyene antibiotic
	7275	Laurdan	Polarity sensitive lipid membrane fluorescent probe; used for imaging lipid rafts
	7538	NBD-PE	Single tail (16:0) phospholipid derivative with a fluorophore (NBD) on the head
	7387	Nile Red	Fluorogenic lipid membrane dye
	7509	NR12S	Fluorogenic lipid membrane dye
	7295	SMCy 5.5	Fluorogenic fluorescent probe for imaging lipid droplets; suitable for use with two-photon microscopy
	5118	SynaptoRed™ C2	Fluorescent dye; stains synaptic vesicles
Fluorescent Lysome Probes	7538	NBD-PE	Fluorescent lipid probe, quantitatively measure phospholipidosis
	7314	Pepstatin A Janelia Fluor® 526	Fluorogenic green-emitting lysosome tracker and stain
Fluorescent Probes for Stem Cells	7419	Kyoto Probe-1	Fluorescent probe that selectively identifies undifferentiated iPS/ES cells
Fluorescent Receptor Probes	6873	DC 271	Fluorescent retinoic acid analog; solvochromatic probe
	5561	IHR-Cy3	Potent fluorescent Smo antagonist
	4577	LDV FITC	Fluorescent ligand for $\alpha_4\beta_1$ (VLA-4)
	6046	Naloxone fluorescein	Fluorescent opioid antagonist; fluorescent-derivative of naloxone (Cat. No. 0599)
	2540	Tocrifluor T1117	Fluorescent cannabinoid ligand; fluorescent form of AM 251 (Cat. No. 1117)
Fluorescent Transporter Probes	0846	Chicago Sky Blue 6B	Potent inhibitor of L-glutamate uptake into synaptic vesicles; dye also used as counterstain in histochemistry
	0845	Evans Blue	Dye for assessing cell viability and blood brain barrier permeability; also EAAT inhibitor and iGluR antagonist
	5200	FFN 102	Selective fluorescent substrate of DAT and VMAT2
	5911	FFN 200	Selective fluorescent VMAT2 substrate
	5043	FFN 206	Fluorescent VMAT2 substrate
	6717	FFN 270	Fluorescent substrate for NET and VMAT2
	3878	FFN 511	Fluorescent substrate for VMAT2
	6065	2-NBDG	Fluorescent glucose uptake indicator
	5168	Rose Bengal	VGlut and VMAT inhibitor; stains exposed epithelium in eye

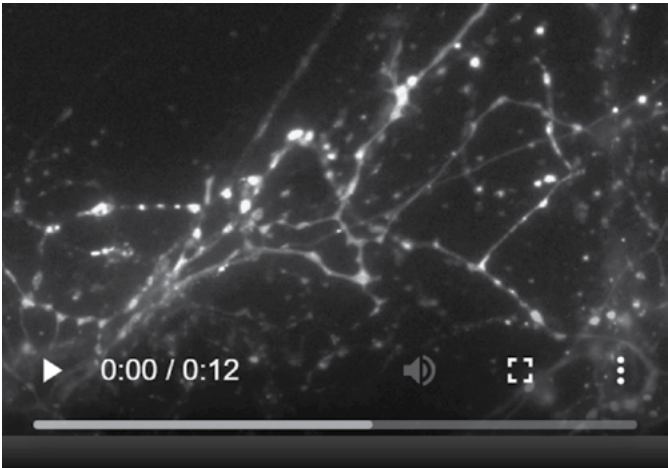


Featured Fluorescent Transporter Probe – FFN 200 dihydrochloride (Cat. No. 5911)

FFN 200 is a selective fluorescent VMAT2 substrate. It selectively traces exocytosis in dopaminergic neuronal cell culture and brain tissue. Excitation and emission maxima are 352 nm and 451 nm, respectively.

Watch FFN 200 in action visit [tocris.com/ffnc-200](https://www.tocris.com/ffnc-200)

Sold with the permission of Columbia University.



**FFN 200 in Cultured Dopaminergic Neurons:** FFN 200 in vesicle clusters in axons of cultured dopaminergic neurons, and exocytosis upon high potassium stimulation (time lapse acquisition at 12 images/min).

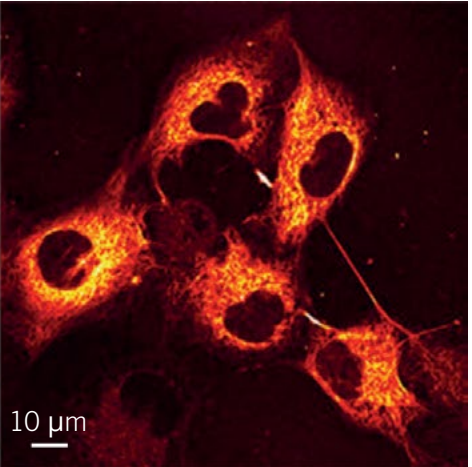
	Cat.No.	Product Name	Description
Histology Stains	5222	Hematoxylin	Histologic stain; used to stain cell nuclei
	3213	Methylene Blue	Biological stain
	5118	SynaptoRed™ C2	Fluorescent dye; stains synaptic vesicles
Microtubule Probes	2226	Flutax 1	Fluorescent taxol derivative; binds microtubules
	6254	Flutax 2	Green fluorescent taxol derivative; binds microtubules
	7315	Taxol Janelia Fluor® 526	Green fluorescent taxol derivative; probe for microtubule staining
	6267	Taxol Janelia Fluor® 549	Yellow fluorescent taxol derivative; probe for microtubule staining; protocol available
	6266	Taxol Janelia Fluor® 646	Red fluorescent taxol derivative; probe for microtubule staining; protocol available

Featured Microtubule Probe – Taxol Janelia Fluor® 646 (Cat. No. 6266)

Taxol Janelia Fluor® 646 is a red fluorescent taxol derivative for direct imaging of the microtubule cytoskeleton. This fluorogenic dye fluoresces only once bound to microtubules, enabling hassle-free no-wash experiments. Excitation maximum = 655 nm; emission maximum = 671 nm.

Protocol available at [tocris.com/taxol-jf646-protocol](https://www.tocris.com/taxol-jf646-protocol)

All images kindly provided by Prof. Christian Soeller, University of Exeter; acquired by Evelina Lucinskaite, Anna Meletiou and Alexander Clowsley.



**Application of Taxol Janelia Fluor® 646, on COS7 cells:** COS7 cells were labeled with 3 μM Taxol Janelia Fluor® 646 for one hour at 37°C. Images were taken on a Leica TCS SP8 Confocal Laser Scanning Microscope.

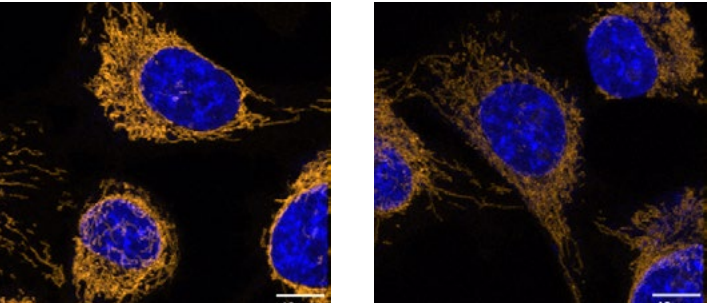
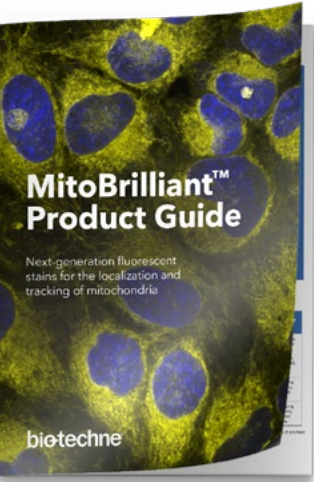
	Cat.No.	Product Name	Description
Mitochondria Dyes and Probes	7700	MitoBrilliant™ 646	Fluorescent mitochondrial probe for live and fixed-cell imaging (red emission)
	7693	MitoBrilliant™ Live 549	Fluorescent mitochondrial probe for live-cell imaging (yellow/orange emission)
	7417	MitoBrilliant™ Live 646	Fluorescent mitochondrial probe for live-cell imaging (red emission)
	7641	Mito-HE	Fluorescent mitochondrial superoxide indicator in live cells
	6444	MitoMark Green I	Green fluorescent mitochondrial stain; cell permeable
	6445	MitoMark Red I	Red fluorescent mitochondrial stain; cell permeable
	4428	MitoPY1	Fluorescent mitochondrial hydrogen peroxide indicator

Featured MitoBrilliant™ Probes

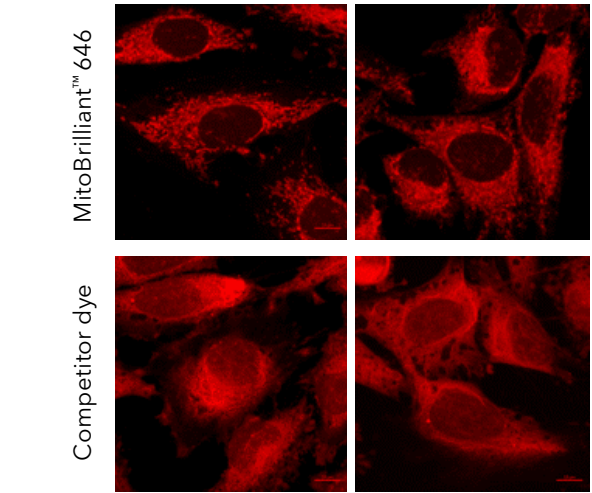
MitoBrilliant™ probes are next-generation fluorescent stains for the localization and tracking of mitochondria in both live- and fixed-cells. The MitoBrilliant range harnesses Janelia Fluor® dye technology, conferring some of the properties of these dyes into mitochondrial stains.

The ‘MitoBrilliant Live’ dyes accumulate in the mitochondria of live cells in a mitochondrial membrane potential ( $\Delta\psi$ m) dependent manner. Upon loss of the mitochondrial membrane potential, the dyes disperse, providing a dynamic assessment of the mitochondrial membrane potential in live cells. Two MitoBrilliant Live dyes are available: MitoBrilliant™ Live 646 (Cat. No. 7417; red emission) and MitoBrilliant™ Live 549 (Cat. No. 7693; yellow/orange emission). They are suitable for use in flow cytometry, imaging and high-content screening.

MitoBrilliant™ 646 (Cat. No. 7700; red emission) is a corresponding probe suitable for both live and fixed-cell staining. It is retained in mitochondria following fixation with exceptionally bright staining and is suitable for use in flow cytometry, imaging, high-content screening and STED super-resolution microscopy.



**Mitochondrial probe brightness comparison:** HeLa cells incubated with 100 nM of MitoBrilliant™ Live 549 (left) or 100 nM of a leading competitor dye (right), for 40 min. Cells were counter-stained with DAPI (Cat. No. 5748). Image taken using an LSM880 Confocal with a 63X objective with the same setting for both images.



**Performance of MitoBrilliant™ 646 after fixation:** HeLa cells incubated with 100 nM of MitoBrilliant™ 646 for 40 min, then fixed for 10 min in 4% PFA. Images taken using an LSM880 Confocal and 63x oil objective. Scale bar = 10 μm.

MitoBrilliant™ Research Product Guide

Our guide highlights the use of MitoBrilliant in different research applications and provides background information on Mitochondria.

To download visit: [tocris.com/literature/product-guides/mitobrilliant](https://www.tocris.com/literature/product-guides/mitobrilliant)

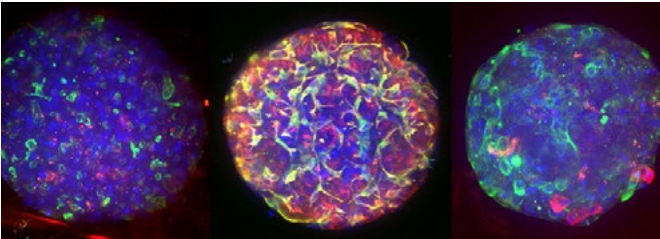
	Cat.No.	Product Name	Description
Neuron & Astrocyte Probes	5146	Sulforhodamine 101	Red fluorescent dye; selective astrocyte marker
	5118	SynaptoRed™ C2	Fluorescent dye; stains synaptic vesicles
Other Fluorescent Probes	5712	4-Ethynyl-N-ethyl-1,8-naphthalimide	Enables imaging of cell surface, intracellular fucosylated glycoproteins and glycolipids
	7391	O-Propargyl-puromycin	Puromycin analog for labeling newly synthesized proteins
SCOTfluor Probes	7446	SCOTfluor 510, fluoro	Amine-reactive fluorescent probe precursor
	7448	SCOTfluor lactic acid probe 510	Fluorescent lactic acid probe

Fluorescence Imaging Reagents

	Cat.No.	Product Name	Description
Anti-Fade Reagents	4055	L-Abscorbic acid	Commonly used antifade reagent in live cell microscopy; naturally occurring antioxidant
	6002	Trolox	Antifade reagent; antioxidant vitamin E derivative; cell permeable and water soluble
Tissue Clearing	7389	Tissue Clearing Pro	Tissue clearing reagent kit
	7563	Tissue Clearing Pro Reagent 1	Tissue clearing reagent
	7564	Tissue Clearing Reagent 2	Tissue clearing reagent
	7390	Tissue Clearing Pro-Organoid	3D cell culture clearing reagent kit
	7565	Tissue Clearing Pro-Organoid Reagent	3D cell culture clearing reagent

Tissue Clearing Pro (Cat. No. 7389) and Tissue Clearing Pro-Organoid (Cat. No. 7390)

Tissue Clearing Pro is an easy-to-use kit, which allows rapid, effective, non-destructive and reversible tissue clearing and staining of whole tissues (Tissue Clearing Pro) or organoids, 3D cell cultures and microtissues (Tissue Clearing Pro-Organoid). Tissue Clearing Pro and Tissue Clearing Pro-Organoid are solvent-based tissue clearing techniques, comparable to BABB and DISCO. Tissue Clearing Pro-Organoid does not cause significant shrinkage or tissue damage and does not use as harsh a solvent as other solvent based techniques.



Key Features of Tissue Clearing Pro and Tissue Clearing Pro-Organoid

- Rapid, easy to use, reversible and non-destructive
- Compatible with fluorescent protein, immunolabeling and small molecule dye staining techniques
- Suitable for use with all standard dyes and buffers
- Following 3D imaging, Tissue Clearing Pro can be reversed for follow-up 2D histology staining
- Tissue Clearing Pro can clear a whole mouse brain of up to 8 mm thickness in 24 hours or 2 hours for 1 mm thick sections
- Tissue Clearing Pro-Organoid allows 3D cell cultures up to 500 µm thick to be cleared in minutes

**Tissue Clearing Pro-Organoid (Cat. No. 7390)** was applied to liver HepaRG spheroids, labeled with DAPI, and structure stained. Left: DNA, MRPII and MDRI; Middle: DNA, CD68, Albumin and Vimentin; Right: DNA, panCK and CD31.

	Cat.No.	Product Name	Description
Bioluminescent Substrates	6823	CycLuc 1	Synthetic luciferase substrate; brain penetrant
	5427	D-Luciferin	Firefly luciferase substrate; cell permeable
	6555	TokeOni	NIR-emission luciferin analog; orally bioavailable and brain penetrant

Substrates for Bioluminescent Imaging

Bioluminescent substrates are commonly utilized for non-invasive monitoring of biological processes, for example in bioluminescence imaging (BLI) in both *in vivo* and *in vitro* settings. Luciferase substrates emit light when they become oxidized, which can be detected using fluorescence microscopy.

Firefly luciferase (Fluc) and D-luciferin

The Firefly luciferase (Fluc) and D-Luciferin BLI system has long been utilized in standard lab techniques such as monitoring tumor growth and intracellular signaling activity *in vitro* and *in vivo*. While this system is very useful for many applications, it is less suitable for experiments requiring detection in deep tissue, because of the relatively short emission wavelength produced by D-Luciferin ( $\lambda_{\text{max}}$  = 562 nm). This wavelength is very similar to those of melanin ( $\lambda_{\text{max}}$  = ~600 nm) and hemoglobin ( $\lambda_{\text{max}}$  = 415-577 nm), making signal detection of D-luciferin emanating from deep within tissue challenging to detect.

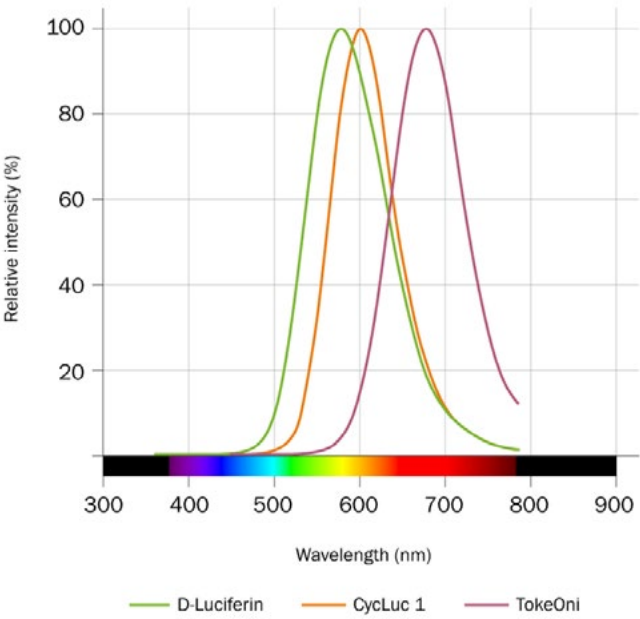
Deep Tissue Bioluminescent Imaging: TokeOni

In bioluminescent imaging, effective deep tissue imaging requires the emission of red-shifted light in the near-IR region.

A major breakthrough towards developing brighter bioluminescence in the desired near-IR region has been the development of engineered luciferases (Akaluc), which permit higher accumulation inside cells with lower toxicity, and modified luciferin substrates such as TokeOni (Cat. No. 6555, also known as AkaLumine HCl) ( $\lambda_{\text{max}}$  = 677 nm). TokeOni allows for deep tissue imaging in the near-IR region and exhibits excellent tissue distribution, including good brain permeability when orally administered.

The improved properties of luciferin substrates combined with engineered luciferases open up many *in vivo* applications including monitoring stem cell fate, tumor growth and metastasis and assessing gene-editing technologies.

Bioluminescent Substrates Emission Spectra

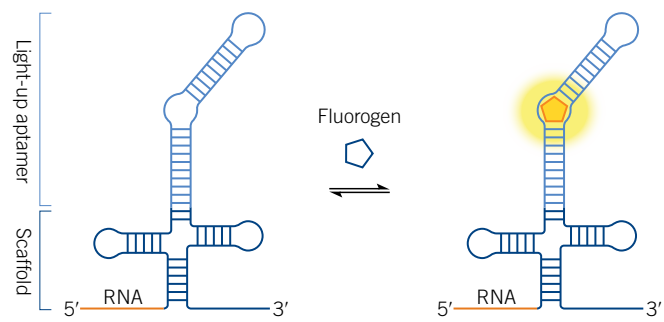


**Emission spectra of bioluminescent substrates:** D-Luciferin, CycLuc 1 and TokeOni span the color spectrum, making them suitable for different applications. TokeOni is well suited to deep tissue *in vivo* applications because of its NIR emission wavelength.



	Cat.No.	Product Name	Description
Aptamer-Based RNA Imaging Reagents	7466	BI dihydrochloride	DFHBI derivative for imaging of RNA in living cells that bind Broccoli aptamers; λabs/λem 470/505 nm.
	5609	DFHBI	GFP fluorophore mimic for imaging RNA in living cells; activated by binding Spinach2 and Broccoli aptamers; λabs/λem 447/501 nm.
	5610	DFHBI 1T	GFP fluorophore mimic for imaging RNA in living cells; activated by binding Spinach2 and Broccoli aptamers; λabs/λem 482/505 nm.
	6434	DFHO	RFP fluorophore mimic for imaging RNA in living cells; activated by binding Corn aptamers; λabs/λem 505/545 nm.
	7277	HBC 530	GFP fluorophore mimic for imaging RNA in live cells; activated by binding to Pepper aptamers; suitable for confocal and two-photon microscopy; λabs/λem 485/530 nm.
	7660	TBI	Fluorogenic ligand for Broccoli RNA aptamer; λabs/λem 485/524 nm

Light-up Aptamer Principles



**Example of Light-up Aptamer Application for Monitoring Gene Expression:** RNA with a light-up aptamer coded (light blue structure) is expressed, a fluorogen (orange pentagon) binds and becomes highly fluorescent. Image adapted from Neubacher and Hennig (2019). PMID: 30102012

Aptamer-based RNA Imaging Technology

Light-up aptamers or Fluorescent light-up aptamers (FLAPs) are a genetically-encoded RNA imaging platform. They are designed to bind specific fluorogenic dyes that ‘light-up’ only in the bound state. This property of ‘fluorogenicity’ means that fluorescence can be ‘switched on’ upon RNA expression. An RNA-based fluorogenic complex or module is made up of two parts, a light-up RNA aptamer, and a fluorogenic cognate dye, the “fluorogen”, which binds the light-up aptamer with high affinity. Once bound, the complex becomes highly fluorescent.

- Light-up Aptamer systems offer several advantages over traditional MS2 and GFP imaging systems:
- Fluorogenic nature produces exceptionally high signal-to-noise ratio
  - Very bright fluorescent signal
  - Light-up aptamers are small RNA tags, thus have a lower propensity to interfere with cellular functions
  - They enable direct, fast measurement of gene transcription at the RNA level, providing a more accurate real time observation of RNA localization and promoter activity; GFP can take up to 30 minutes after stimulation to be translated into protein.

	Cat.No.	Product Name	Description
Fluorescent Probes for Imaging Bacteria	7506	Click <i>N</i> -Acetylmuramic acid - azide	Bacterial peptidoglycan derivative; suitable for ‘click’-conjugation to fluorescent dyes
	7714	EDA-DA	Unnatural dipeptide building block with alkyne group for functionalizing peptidoglycan
	6647	HADA	Blue fluorescent D-amino acid, λabs/λem 405\450 nm, for labeling peptidoglycans in live bacteria
	6648	NADA-green	Fluorescent D-amino acid, λabs/λem 450\555 nm, for labeling peptidoglycans in live bacteria
	7408	OGDA	Green fluorescent D-amino acid. Compatible with imaging bacteria using STED microscopy
	6649	RADA	Orange-red TAMRA-based fluorescent D-amino acid, λabs/λem 554\580 nm, for labeling peptidoglycans in live bacteria
	7406	Rf470DL	Blue rotor-fluorogenic fluorescent D-amino acid for labeling peptidoglycans in live bacteria
	6659	sBADA	Green fluorescent D-amino acid, λabs/λem 490\510 nm, for labeling peptidoglycans in bacteria
	6802	6 TMR Tre	Fluorescent trehalose, λabs/λem 532\580 nm; selectively labels mycobacterial cell envelope
	6650	YADA	Green-yellow lucifer yellow-based fluorescent D-amino acid, λabs/λem 426\535 nm, for labeling peptidoglycans in live bacteria

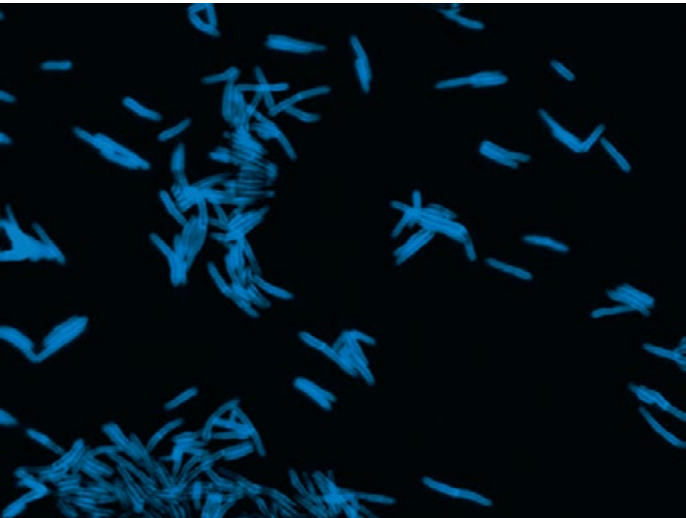
FDAAs Probes for Imaging Bacteria

Fluorescent probes for imaging bacteria are useful for advancing bacterial research and antibiotic design. Fluorescent D-amino acids (FDAAs) are an essential tool for studying peptidoglycan synthesis and dynamics, and our range now spans the visible light spectrum giving you more options for multiplexing. FDAAs efficiently label peptidoglycans in bacterial cell walls *in situ*, allowing you to investigate and visualize cell morphology and formation, as well as bacterial growth. They can be used in gram-negative and gram-positive bacteria, and they are suitable for use with super-resolution microscopy (SRM).

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Labeling of active peptidoglycan synthesis in cell wall of growing mycobacteria.



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A Direct Way to Detect Target Molecules by Flow Cytometry

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	Cat.No.	Product Name	Description
TSA Vivid™ Fluorophore Kits	7523	TSA Vivid™ Fluorophore Kit 520	Signal amplification kit for use in ICC, IHC, FISH
	7526	TSA Vivid™ Fluorophore Kit 570	Signal amplification kit for use in ICC, IHC, FISH
	7527	TSA Vivid™ Fluorophore Kit 650	Signal amplification kit for use in ICC, IHC, FISH

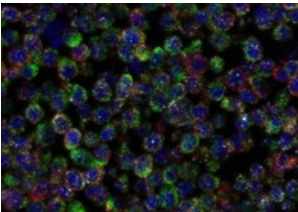
TSA Vivid™ Fluorophore Kits

TSA Vivid™ Fluorophore Kits increase brightness and improve performance in ICC, IHC and FISH applications. They are specifically designed for exceptional signal-to-noise performance in the RNAscope™ Multiplex Fluorescent v2 Assay, enabling visualization of gene expression at the single cell level.

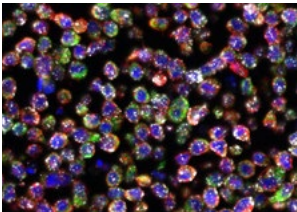
TSA Vivid™ Fluorophore Kits Key features and Applications

- Brighter than equivalent competitor fluorophores
- Delivers leading performance with the RNAscope Multiplex Fluorescent v2 Assay
- Suitable for multiplexing
- Can be combined with DAPI counter-staining

Leading Competitor



TSA Vivid™



The images show 3-plex RNAscope Multiplex Fluorescent v2 Assay plus DAPI counter-stain (Cat. No. 5748) on HeLa cells with TSA Vivid™ dyes (520, 570, 650, right) and the corresponding leading competitor dyes (left). All dyes were used at 1:1500 dilution. Markers shown are Polr2a in green, PPIB in red and UBC in white.

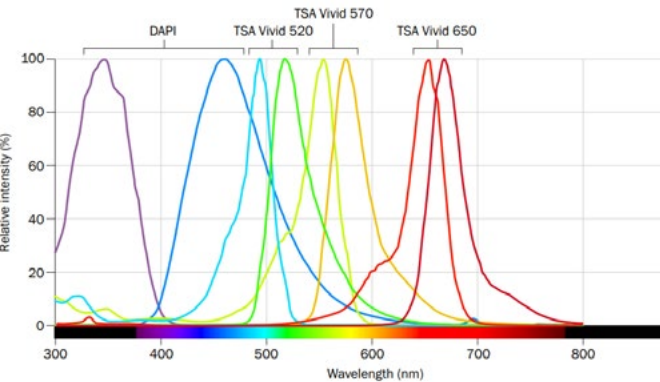
What researchers are saying about TSA Vivid™



**We tested the Tocris TSA Vivid™ dyes in our RNAscope multiplex fluorescence assay and were very pleased to find that they slotted into the protocol with no changes necessary. No optimization was required, and we were able to substitute our usual dyes at the same concentration. We are impressed with the results and how bright the dyes are.”**

- Dr. Julia Jones, Senior Scientific Officer, Cancer Research U.K.

Optical data for DAPI, TSA Vivid™ 520, 570 and 650



# Visualize Multiple Markers Simultaneously in Tissue

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	Cat.No.	Product Name	Description
Tyramide Signal Amplification (TSA) Reagents	6241	Biotinyl Tyramide	Reagent widely used for signal amplification in IHC and FISH
	6457	Cyanine 3 Tyramide	Orange fluorescent reagent widely used for signal amplification in IHC and FISH
	6458	Cyanine 5 Tyramide	Red fluorescent reagent widely used for signal amplification in IHC and FISH
	7236	Digoxigenin Tyramine	Reagent used for Tyramide Signal Amplification in IHC, ICC and FISH
	6456	Fluorescein Tyramide	Green fluorescent reagent widely used for signal amplification in IHC and FISH

TSA Reagents for Enhancing IHC, ICC & FISH Signals

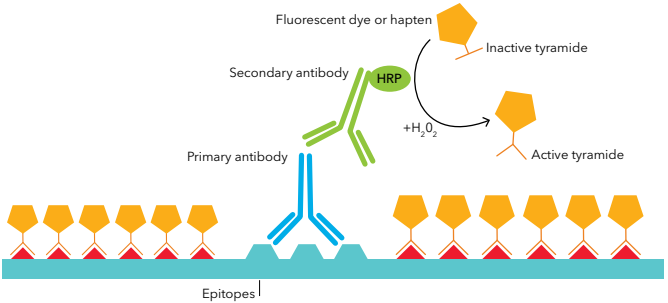
Tyramide Signal Amplification (TSA), also known as Catalyzed Reporter Deposition (CARD), offers an effective way to efficiently enhance signal and detection capabilities for low-abundance targets in immunocytochemistry (ICC), immunohistochemistry (IHC), and *in situ* hybridization (ISH) applications.

Tyramide Signal Amplification Principles

A primary and secondary antibody are used to label a tissue or cell sample. The secondary antibody is pre-conjugated to horseradish peroxidase (HRP), which in the presence of H<sub>2</sub>O<sub>2</sub>, catalyzes a labeled tyramide substrate into a highly reactive species that covalently binds to tyrosine residues on the proteins in close proximity to the antibodies and HRP, thus providing signal amplification.

Key Features of Tyramide Signal Amplification

- Allows detection of low-abundance targets
- Enhances signals in IHC, ICC and FISH
- Reduces the amount of primary antibody required
- 100-fold more sensitive than conventional methods
- Simple, flexible, and easy to incorporate into IHC, ICC and FISH workflows
- Compatible with fluorescent multiplex systems



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