

Better images with Nanobodies

Nano-Secondaries[®], Nano-Boosters, Nano-Labels, Chromobodies[®], and Nanobody-Fc fusions for immunofluorescence



About Us

Since its inauguration in 2008, ChromoTek, from its base in Martinsried near Munich, Germany, has pioneered the development and commercialization of Nanobody-based research reagents. Its premium Nanobody-based tools provide a higher level of performance than conventional IgG antibodies. ChromoTek is the global market and product leader in high-quality and reliable Nanobody-based reagents, which assist our customers' research. In addition, ChromoTek is a trusted service provider of custommade Nanobodies for the pharmaceutical industry.

ChromoTek's mission is to support extraordinary discoveries with high-performing Nanobody-based affinity reagents in proteomics and cell biology. ChromoTek strives to improve, accelerate, and simplify our customers' research around the world. ChromoTek has been part of Proteintech Group since 2020. Proteintech Group, founded in 2002 and headquartered in Rosemont, IL, is a leading manufacturer of Proteintech antibodies and ELISAs plus HumanKine cytokines and growth factors. The Proteintech Group has the largest proprietary portfolio of self-manufactured antibodies covering more than 2/3 of the human proteome. With over 100,000 publications and confirmed specificity, Proteintech Group offers antibodies and immunoassays across research areas. In addition, Proteintech produces HumanKine cytokines, growth factors, and other proteins that are human cell line expressed, highly bioactive, and GMP-grade. It is ISO13485 and ISO9001-2015 accredited.

Cover:

Upper left: Confocal image of stained HeLa cells (mouse lgG1 anti-Vimentin, mouse lgG2b anti-Lamin, mouse lgG3 anti-MOT detected with Nano-Secondaries[®]).

Upper right: STED super-resolution imaging of Spot-tagged Actin-Chromobody® with Spot-Label® ATTO 594.

Lower left: Confocal image of HeLa cells transiently transfected with Tom70-EGFP and immunostained with GFP-Booster Alexa Fluor[®] 488 (green). Nuclei were stained with DAPI (blue).

Lower right: Confocal image of stained HeLa cells (rabbit IgG anti-Lamin, mouse IgG1 anti-COX4, mouse IgG2b anti-Tubulin detected with Nano-Secondaries[®]).

Imaged at the Core Facility Bioimaging at the Biomedical Center, LMU Munich, Germany.



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Better images with Nanobodies



Nanobody (dark green), fluorescent dyes (red), conventional antibody and antibody domains (dark blue), and Green Fluorescent Protein (GFP, light green).

ChromoTek has developed a suite of superior reagents specialized for certain imaging applications using the advantages of its tailor-made Nanobodies.

Nanobodies, also termed VHHs, are the smallest known antibodies. Due to their small size, high specificity, low background, and consistently high quality, they enable better performance in immunofluorescence (IF). ChromoTek Nanobody-based research reagents for imaging consist of

- **Nano-Secondaries**[®]: Nanobodies conjugated to fluorescent dyes binding to conventional antibodies.
- **Chromobodies**[®]: Nanobodies fused to fluorescent proteins. They are optimized for live-cell imaging.
- **Nano-Boosters** and **Nano-Labels**: Nanobodies conjugated to fluorescent dyes. Nano-Boosters bind to fluorescent proteins, Nano-Labels bind to non-fluorescent proteins or peptides.
- **Nanobody-Fc fusions**: Nanobodies fused to Fc-binding domains.



A size comparison of a Nanobody (green), a conventional antibody (dark blue), and a complex of primary (dark blue) and secondary (light blue) antibodies.

ChromoTek Nanobody advantages in imaging

I. Small size of Nanobodies

Nanobodies/VHHs are single-domain antibody fragments with a molecular weight of only 15 kDa and 2 nm in size. They are the smallest known antibodies and are ideal for immunofluorescence assays.

Vimentin in HeLa cells imaged with Leica GSDIM system. The cells were stained with monoclonal mouse IgG1 anti-Vimentin antibody and Nano-Secondary[®]*alpaca anti-mouse IgG1, recombinant VHH, Alexa Fluor*[®]*488 [CTK0103, CTK0104]. Courtesy of Dr. Leila Nahidiazar, Dr. Jop Kind, and Prof. Kees Jalink, from the Hubrecht Institute and The Netherlands Cancer Institute, Netherlands. Scale bar, 1000 nm.*



II. Better tissue penetration

Nanobodies enable better and faster tissue penetration in crowded cellular environments and tissues as they are 10 times smaller than conventional IgG antibodies. Especially in immunostainings of tissues, organs, or whole animals, the penetration rate directly influences image quality and incubation time.

III. Minimal label displacement

The small size of Nanobodies results in a very short distance between the epitope and fluorescent dye. There is minimal epitope-label displacement with fluorescently labeled Nanobodies. For Nano-Boosters and Nano-Labels the epitope-label displacement is about 2 nm. Therefore, Nanobodies reduce the localization error between the dye and the epitope and increase the image resolution.



The smaller the better - Nanobodies, e.g., Nano-Boosters penetrate tissue better than conventional antibodies: The comparison of conventional anti-GFP antibody and GFP-Booster shows the superior tissue penetration rate of GFP-Booster. Fluorescent images of transgenic mouse tissue expressing Cx3Cr1-EGFP. EGFP signal was enhanced either with conventional anti-GFP antibody (top image) or with GFP-Booster (bottom image).





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Primary & Secondary Antibodies >300 kDa

Epitope to label displacement: Schematic comparison of a Nanobody (green) conjugated to fluorescent dyes (red) vs. a conventional primary antibody (dark blue) and secondary antibodies (light blue) conjugated to fluorescent dyes. Epitope is shown in pink.



Distance between the epitope and fluorophore with a labeled Nanobody, a labeled primary IgG antibody, and a complex of primary IgG antibody and labeled secondary IgG antibodies.



IV. Reproducibility and validation

All ChromoTek Nanobodies including Nano-Secondaries, Nano-Boosters, Nano-Labels, Chromobodies, and Nanobody-Fc fusions are thoroughly characterized and tested for high specificity, high affinity, and low background. ChromoTek Nanobodies are validated by genetic approach, meaning they are tested in their target application on cell lines that express and do not express their tag and/or are benchmarked with established conventional antibodies.

All Nanobodies are recombinantly expressed. Together with our strict QC, we guarantee consistently high quality with almost no lot-to-lot variation for reproducible results. All Nanobody-based reagents are monoclonal unless otherwise stated.

V. Fluorescent labeling

ChromoTek has developed proprietary labeling protocols for Nanobodies. ChromoTek Nanobodies have a constant degree of labeling for higher reproducibility and reliability. Most Nano-Secondaries, Nano-Boosters, and Nano-Labels have a labeling efficiency greater than 95% and they are available with bright Alexa Fluor dyes or ATTO dyes. These provide strong signals for confocal and standard microscopy and offer flexible solutions for super-resolution microscopy including STED and STORM.



Nano-Secondaries: The next level of secondary antibodies

Nano-Secondaries are a novel class of secondary antibodies for higher resolution, cleaner images, and faster immunostaining. Nano-Secondaries are monovalent and consist of Nanobodies/VHHs that bind to primary antibodies with high affinity in a species- and isotype-specific manner. Nano-Secondaries are conjugated to Alexa Fluor dyes. Along with immunofluorescence, they can be used in Western blotting and flow cytometry.



Left: primary antibody (dark blue) & Nano-Secondary (green) complex Right: complex of primary and polyclonal secondary antibodies (light blue). Epitope is shown in pink and conjugated fluorescent dyes are shown in red.

I. Co-incubation to save time

ChromoTek Nano-Secondaries are monovalent and enable one-step immunostaining: the simultaneous incubation of the sample with primary antibodies and Nano-Secondaries.. This saves incubation time, reduces washing steps and hands-on time, and results in gentle incubation of the sample. Co-incubation provides the same high image quality as sequential incubation of primary and secondary antibodies.

One-step co-incubation with Proteintech primary antibodies and Chromotek Nano-Secondaries.

Total time 100 min.

1. Fix, permeabilize and block 2. Incubation with conventional primary and Nano-Secondary 1.5 hr at room temperature 3. Wash 3x (10 min) 4. Detect

B. Traditional workflow with conventional primary and secondary antibodies.

Total time 170 min.

1. Fix, permeabilize and block 2. Incubate with primary antibody (1.5 hr) 3. Wash 3x (10 min) 4. Incubate fluorescently labeled secondary antibody (1 hr) 5. Wash 3x (10 min) 6. Detect



Workflow comparison of a time-saving one-step immunostaining using primary antibodies and Nano-Secondaries vs. immunostaining using primary and conventional secondary antibodies.



Immunoblot showing no cross-reactivity from Nano-Secondaries to IgGs from commonly used species & isotypes. Note: competitor's cross-reactivity despite pre-adsorption against mouse IgGs (lowest line).

II. No cross-reactivity

Nano-Secondaries are isotype-specific. There is no cross-reactivity to antibodies and sera from commonly used species and isotypes. During development, we select only Nano-Secondaries with the desired specificity, i.e., cross-reactive and low-specific Nanobodies are discarded. Unlike traditional (polyclonal) secondary antibodies, where each batch has to be depleted and still contains fractions that bind to other isotypes' and/or species' antibodies, Nano-Secondaries are recombinantly produced and hence have no lot-to-lot variation meaning they permanently retain their excellent properties..



Secondary igo Antibody

In contrast to conventional secondary antibodies, Nano-Secondaries do not contain a conserved Fc-domain that can be bound by endogenous Fc-receptors, which would result in unspecific background staining.

III. Low background

In addition to high isotype specificity, Nano-Secondaries show very low background staining. Only Nano-Secondaries with high specificity, high sensitivity, and low background are selected during development to minimize off-target effects. Accordingly, they detect isotype-specific IgGs only and do not unspecifically bind to other cellular components. Since Nano-Secondaries do not contain an Fc-domain, they cannot be bound to endogenous Fc-receptors. This is another reason for their low background and excellent signal-to-noise ratio.

IV. Multiplexing

Simultaneous detection of multiple proteins on a single sample requires (i) different primary antibodies that bind to specific proteins and (ii) secondary antibodies or Nano-Secondaries that bind to and can distinguish between these primary antibodies. Due to their high isotype specificity and the absence of cross-reactivity, Nano-Secondaries bind only to primary antibodies of a specific isotype. Even co-incubation of multiple primaries along with multiple Nano-Secondaries is possible. Nano-Secondaries are also compatible with conventional secondary antibodies in multiplexing assays.

Multiplexing of HeLa cells with Nano-Secondaries alpaca anti-mouse and anti-rabbit. Yellow: rabbit IgG anti-Lamin + Nano-Secondary alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor 568 [CTK0101, CTK0102], green: mouse IgG1 anti-COX4 + Nano-Secondary alpaca anti-mouse IgG1, recombinant VHH, Alexa Fluor 488 [CTK0103, CTK0104], magenta: mouse IgG2b anti-Tubulin + Nano-Secondary alpaca anti-mouse IgG2b, recombinant VHH, Alexa Fluor 647 [CTK0105, CTK0106]. Confocal images were acquired with a Leica TCS SP8 microscope, 100x oil objective, and deconvolved with Huygens Professional (SVI). Images were recorded at the Core Facility Bioimaging at the Biomedical Center, LMU Munich, Germany. Scale bar, 10 µm.





HeLa cells were immunostained with rabbit IgG anti-Lamin B1 antibodies and Nano-Secondary alpaca anti-human IgG/antirabbit IgG, recombinant VHH, Alexa Fluor 568 [CTK0101, CTK0102] (1:1,000). Confocal and gated STED images were acquired with a Leica TCS SP8 STED 3X microscope, pulsed depletion with a 775 nm laser. Images were recorded at the Core Facility Bioimaging at the Biomedical Center, LMU Munich, Germany. Scale bar, 2 µm.

V. Super-resolution microscopy

Due to their small size, Nano-Secondaries are excellent tools for super-resolution microscopy, e.g., STED, STORM. Using Nano-Secondaries it is possible to increase the resolution of images. Nano-Secondaries are offered with different bright and stable Alexa Fluor dyes: Alexa Fluor 488, Alexa Fluor 568, and Alexa Fluor 647.

Nano-Boosters and Nano-Labels: Fluorescent probes for immunofluorescence

ChromoTek Nano-Boosters and Nano-Labels are pre-conjugated fluorescent probes that enable higher image quality in epifluorescence, confocal, and super-resolution microscopy. Nano-Boosters stabilize, enhance, and reactivate the signal of fluorescent proteins (GFP- and RFP-Boosters), while Nano-Labels fluorescently label endogenous cellular proteins (Vimentin- and Histone-Label) or the Spot-Tag[®] (Spot-Label[®]).



Schematic representation of a Nano-Booster or Nano-Label (left) versus a primary and polyclonal secondary antibody complex (right).

I. Enhancing, stabilizing, and reactivating the signal of fluorescent proteins

Fluorescent proteins (FPs) are powerful tools to study protein localization and dynamics in living cells. However, genetically encoded FPs have several disadvantages compared to chemical dyes:

- The signal intensities of fixed samples from cells expressing FP fusion at physiological expression levels are usually very low.
- Neither the photostability nor quantum efficiency of FPs is generally sufficient for super-resolution microscopy (e.g., 3D-SIM, STED or STORM/PALM).
- Many cell biological methods such as HCl treatment for BrdU-detection, the EdU-Click-iT[™] treatment, or heat denaturation for FiSH lead to disruption of the FP signal.

GFP-Booster and RFP-Booster stabilize, enhance, and reactivate the fluorescent signals of FPs such as GFP, RFP, and mCherry in immunofluorescence. They are monovalent and are offered labeled with various Alex Fluor dyes or ATTO dyes.



HeLa cells transiently transfected with PCNA-mRFP and Tom 70-EGFP were subjected to one-step immunostaining with RFP-Booster Alexa Fluor 568 (red) and GFP-Booster Alexa Fluor 488 (green). Images were recorded at the Core Facility Bioimaging at the Biomedical Center, LMU Munich, Germany. Scale bar, 10 μm.





Monoclonal rabbit anti-H2A IgG / anti-rabbit Alexa Fluor 568

Merge Histone-Label / anti-H2A lgG antibody

ChromoTek Histone-Label is a convenient, ready-to-use, and high-performing chromatin staining probe with low background levels that differentiates between euchromatin and heterochromatin. HeLa cells stained in parallel with Histone-Label and a monoclonal rabbit anti-H2A IgG/ anti-rabbit Alexa Fluor 568 secondary antibody. Histone-Label co-localizes with conventional antibody staining; however, Histone-Label better penetrates into the tightly packed nuclei than the anti-H2A IgG and secondary IgGs, which are one order of magnitude larger than the Histone-Label: see more green signal from Histone-Label at the center of the nuclei and more red signal from anti-H2A IgG on the surface/edge of the nuclei in the merged image (lower right). Scale bar, 10 µm.

II. Direct immunostaining of endogenous proteins

Nano-Labels precisely stain proteins in their cellular environment. Due to their minimal epitope-label displacement and the superior accessibility to epitopes in crowded cellular/organelle environments, Nano-Labels enable high resolution in immunofluorescence and microscopy. The Vimentin-Label specifically binds to the vimentin intermediate filament protein. The Histone-Label is a very specific probe for the direct immunostaining of histones, chromosomes, and nuclei as it binds to Histone H2A-H2B heterodimers. Compared to conventional anti-Histone antibodies, the significantly smaller Histone-Label better penetrates into the nucleus and provides more precise staining (see legend).

III. Peptide tag-specific Nanobody for IF: Spot-Label

The Spot-Label is the first Nanobody to be used in superresolution microscopy for the detection of a peptide tag. The Spot-Label specifically detects the Spot-Tag, which can be genetically fused to any protein. Due to its high specificity, high affinity, and the lack of the Fc-domain, the background of the Spot-Label is very low. As with all Nano-Boosters and Nano-Labels, the small size of the Spot-Label allows for higher image resolution. The Spot-Tag is a 12 amino acid peptide tag (PDRVRAVSHWSS); it is easy to clone, has no biological function (inert), and has been tested in various distinct organisms such as plants and human cells. The Spot-Label detects the Spot-Tag at N-terminal, C-terminal, and internal positions.



STED super-resolution imaging of Spot-tagged Actin-Chromobody with Spot-Label ATTO 594 (1:1,000). Gated STED images were acquired with a Leica TCS SP8 STED 3X microscope with pulsed White Light Laser excitation at 590 nm and pulsed depletion with a 775 nm laser. Objective: 100x Oil STED White, NA: 1.4. Pixel size: 21 x 21 nm; z-Step size of z-Stacks: 0.16 µm. Images were deconvolved with Huygens Professional (SVI). STED images were recorded at the Core Facility Bioimaging at the Biomedical Center, LMU Munich, Germany. Scale bar, 10 µm.

Chromobodies: Superior tools for live-cell imaging

Chromobodies are small intracellular functional antibodies that work as fluorescent live-cell nanoprobes. They are optimized for real-time and live-cell imaging of endogenous proteins. Chromobodies are Nanobodies that are genetically fused to a fluorescent protein such as GFP. They are available as DNA plasmids that are transiently transfected into cells. Also, stable cell lines or transgenic organisms can be created.



Scheme of a cell expressing Chromobodies upon transfection with the Chromobody DNA plasmid. The Chromobody plasmid codes for the Nanobody that is genetically fused to GFP. After transfection, the Chromobody is expressed within the cell: The Nanobody part binds to the epitope and the GFP can be detected with a microscope. Here, Lamin-Chromobodies are shown binding to the nuclear Lamina.



Time series of imaging DNA damage in HeLa cells after microirradiation: PARP1-Chromobody allows monitoring of DNA damage after microirradiation in real time in living cells. HeLa cells were subjected to confocal imaging upon laser microirradiation. The white triangle shows the location of the microirradiation. Time-lapse analysis shows the recruitment of PARP1 to DNA damage. Scale bar, 5 μm.

I. Real-time imaging

Upon transfection, the Chromobody protein is intracellularly expressed, binds to the endogenous protein, and, therefore, brings the fluorescent protein to the target structure. Chromobodies can be applied to monitor protein dynamics or visualize biomarkers in secondary screens in real time in live cells.

00:00	+ cyto D 00:06	+ cyto D 00:12
+ cyto D 00:18	+ cyto D 01:00	recovery 00:06
recovery 00:12	recovery 00:30	recovery 04:00

The time series reveals the reorganization of actin after treatment with Cytochalasin D: HeLa cells were subjected to confocal imaging upon treatment with 2 μ M of Cytochalasin D for 1 hr and recovery for 4 hr. Actin-Chromobody enables monitoring of actin dynamics in real time in living cells. Scale bar, 10 μ m.

II. Minimal interference with target function

Chromobodies non-invasively label endogenous proteins without interfering with protein function. This makes them perfect nanoprobes for cellular research and high content analysis. For example, the Actin-Chromobody has been thoroughly compared with multiple alternative Actin labeling methods and was ranked the least interfering probe for the detection of Actin (Actin visualization at a glance. Melak et al. J Cell Sci 2017). Chromobodies do not affect cell viability or migration, intercalate in DNA, influence cellular functions, or show cytotoxic effects when overexpressing fluorescent fusion proteins.

Nanobody-Fc fusions: The Nanobody advantage combined with traditional IgG antibodies

Nanobodies can also be fused to Fc-domains to generate chimeric heavy chain antibodies. The Fc-domain fusion format combines the advantages of Nanobodies with those of traditional antibodies: The Nanobody-Fc fusions (i) are bivalent so they have even higher affinities due to an avidity effect, (ii) bind unique, 3-dimensional conformations of epitopes not recognized by traditional antibodies, and (iii) can be detected and captured using tools from the broad range of antibody reagents like secondary antibodies and Nano-Secondaries. Nanobody-Fc fusions are available with rabbit IgG or mouse IgG1 Fc-domains. Like all ChromoTek Nanobody reagents, Nanobody-Fc fusions are recombinantly expressed.



Schematic representation of a Nanobody-Fc fusion (Nanobody: dark green, Fc-domain: blue) bound by Nano-Secondaries (light green). Epitope is shown in pink and conjugated fluorescent dyes are shown in red.. Of course, Nanobody-Fc fusions can also be detected using traditional secondary antibodies.

I. Increased flexibility

Nanobody-Fc fusions consist of Nanobodies, binding to unique epitopes that traditional antibodies do not recognize, and Fc-domains from traditional antibodies. They have a bivalent structure and can be detected by Nano-Secondaries or conventional secondary antibodies, which results in high signal amplification. For increased flexibility, Nanobody-rabbit IgG Fc fusions and Nanobody-mouse IgG1 Fc fusions are available.



MDCK cells were immunostained with Vimentin recombinant antibody, Nanobody-rabbit IgG Fc fusion [CTK0211] (vfrb, 1:1,000), and Nano-Secondary alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor 647 [CTK0101, CTK0102] (1:1,000). Scale bar, 10 μm.



What is a Nanobody? What are Nano-Secondaries, Nano-Boosters, Nano-Labels, Chromobodies, and Nanobody-Fc fusions?

Camelids such as camels, llamas, and alpacas possess an immune repertoire of three isotype IgG antibodies: IgG1, IgG2, and IgG3. IgG1 is a conventional IgG composed of two heavy chains and two light chains. IgG2 and IgG3 are heavy-chain-only IgG antibodies (HCAbs) that can be distinguished by their hinge regions. These HCAbs lack the CH1 domain of the heavy chain and are devoid of any light chain. The binding domain of a heavy-chain-only IgG is called a Nanobody or VHH. Nanobodies have excellent binding properties and can be recombinantly expressed at consistently high quality with no batch-to-batch variation.

Nano-Secondaries consist of Nanobodies conjugated to fluorescent dyes. Nano-Secondaries bind to IgG antibodies.

Nano-Boosters and **Nano-Labels** are Nanobodies conjugated to fluorescent dyes. Nano-Boosters bind to fluorescent proteins such as GFP, RFP, or mCherry whereas Nano-Labels bind to non-fluorescent proteins or peptide tags.

Chromobodies are Nanobodies that are genetically fused to a fluorescent protein such as GFP. Normally, cells/organisms are transfected with Chromobody DNA plasmids coding for the Chromobody, which is then intracellularly expressed.

Nanobody-Fc fusions are Nanobodies conjugated to either rabbit IgG or mouse IgG1 Fc-domains. They are also called chimeric heavy chain antibodies.

All ChromoTek Nanobodies are recombinantly expressed and the manufacturing process is entirely animal-free.

Our Products

ChromoTek Nanobody-based reagents for imaging:

Nano-Secondaries	Nano-Booster & Nano-Label	Chromobodies	Nanobody-Fc fusions
Nano-Secondary alpaca anti-mouse lgG1	GFP-Booster	Actin-Chromobody	GFP recombinant antibody, VHH-mouse IgG1 Fc fusion
Nano-Secondary alpaca anti-mouse lgG2b	RFP-Booster	Nuclear Actin- Chromobody	GFP recombinant antibody, VHH-rabbit lgG Fc fusion
Nano-Secondary alpaca anti-mouse lgG3	Spot-Label	Cell Cycle-Chromobody	mNeonGreen recombinant antibody, VHH-mouse lgG1 Fc fusion
Nano-Secondary alpaca anti-rabbit lgG	Histone-Label	Dnmt1-Chromobody	mNeonGreen recombinant antibody, VHH-rabbit lgG Fc fusion
Nano-Secondary alpaca anti-human lgG	Vimentin-Label	Histone-Chromobody	TurboGFP recombinant antibody, VHH-mouse lgG1 Fc fusion
		Lamin-Chromobody	TurboGFP recombinant antibody, VHH-rabbit lgG Fc fusion
		PARP1-Chromobody	Vimentin recombinant antibody, VHH-mouse lgG1 Fc fusion
		Vimentin-Chromobody	Vimentin recombinant antibody, VHH-rabbit IgG Fc fusion

Specificity

The GFP-Booster and RFP-Booster bind to the following derivatives of fluorescent proteins:

GFP-Booster	AcGFP, mClover, EGFP, Monomeric EGFP A206K, GFP, GFP S65T, mPhluorin, PA-GFP, Superfolder GFP, TagGFP, TagGFP2 CFP, YFP, Citrine, ECitrine, EYFP, Venus, Ypet
RFP-Booster	mCherry, mKate2, mPlum, mRFP, mRFPruby

Fluorescent dyes

Depending on the product, we offer the following Alexa Fluor and ATTO dyes:

Alexa Fluor	Alexa Fluor 488, Alexa Fluor 568, Alexa Fluor 647
ATTO	ATTO 488, ATTO 594, ATTO 647N

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Ordering information

To place an order visit **ptglab.com** or **chromotek.com**



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