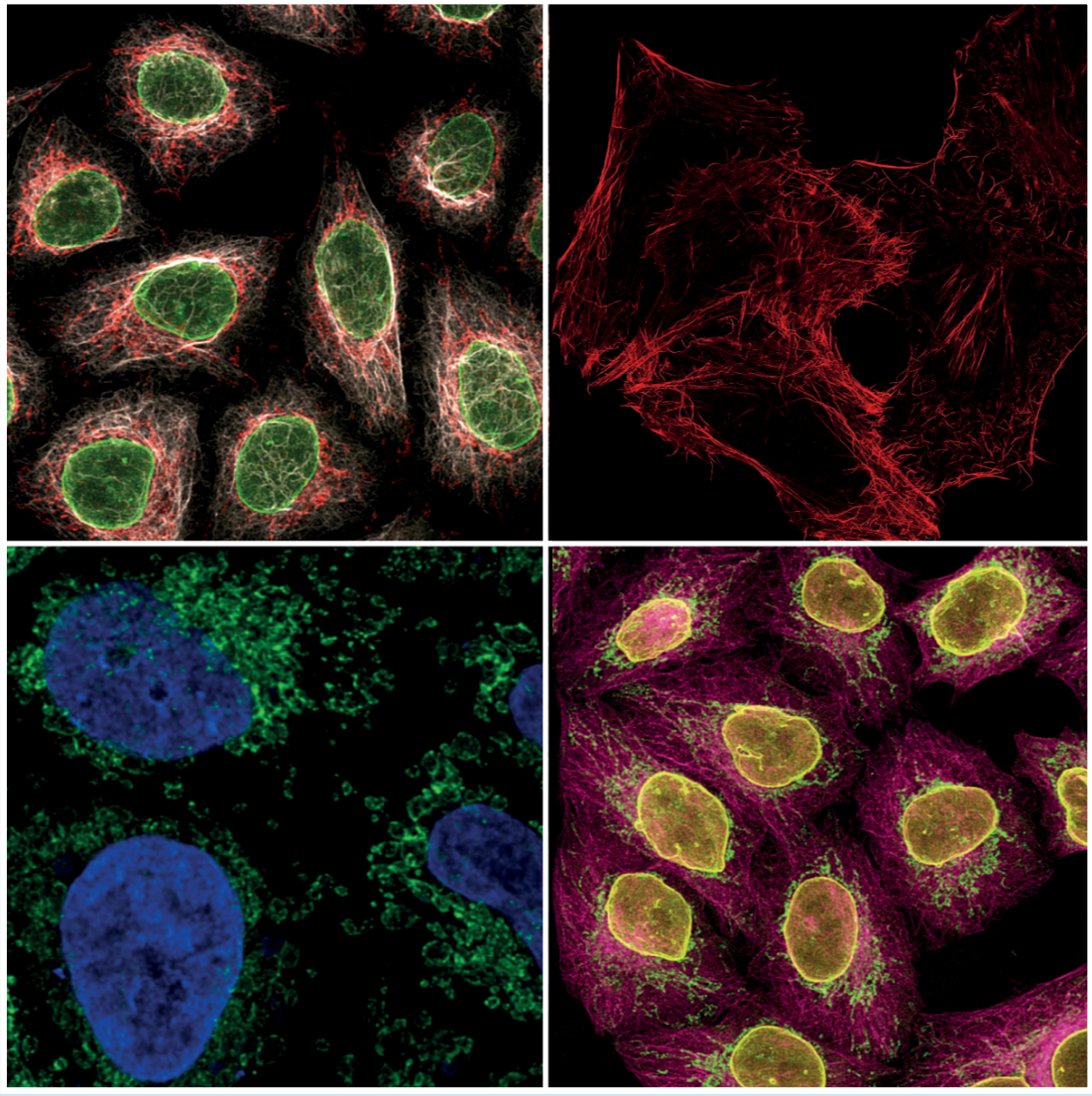
proteintech®

Better Images with chromotek® Nanobodies



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ON THE COVER:

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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).*

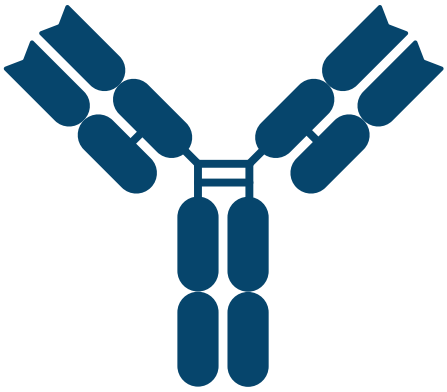
Lower right: *Confocal image of stained HeLa cells with rabbit IgG anti-Lamin, mouse IgG1 anti-COX4, mouse IgG2b anti-Tubulin detected with Nano-Secondary® Reagents.*

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

WHAT IS A NANOBODY?

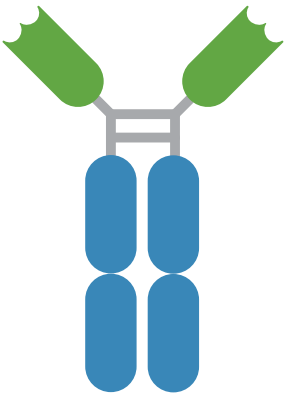
Camelids such as camels, llamas, and alpacas possess an immune repertoire of three isotype IgG antibodies: IgG1, IgG2, and IgG3. IgG1 is a conventional IgG composed of two heavy chains and two light chains. IgG2 and IgG3 are heavy-chain-only IgG antibodies (HCAbs) that can be distinguished by their hinge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

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



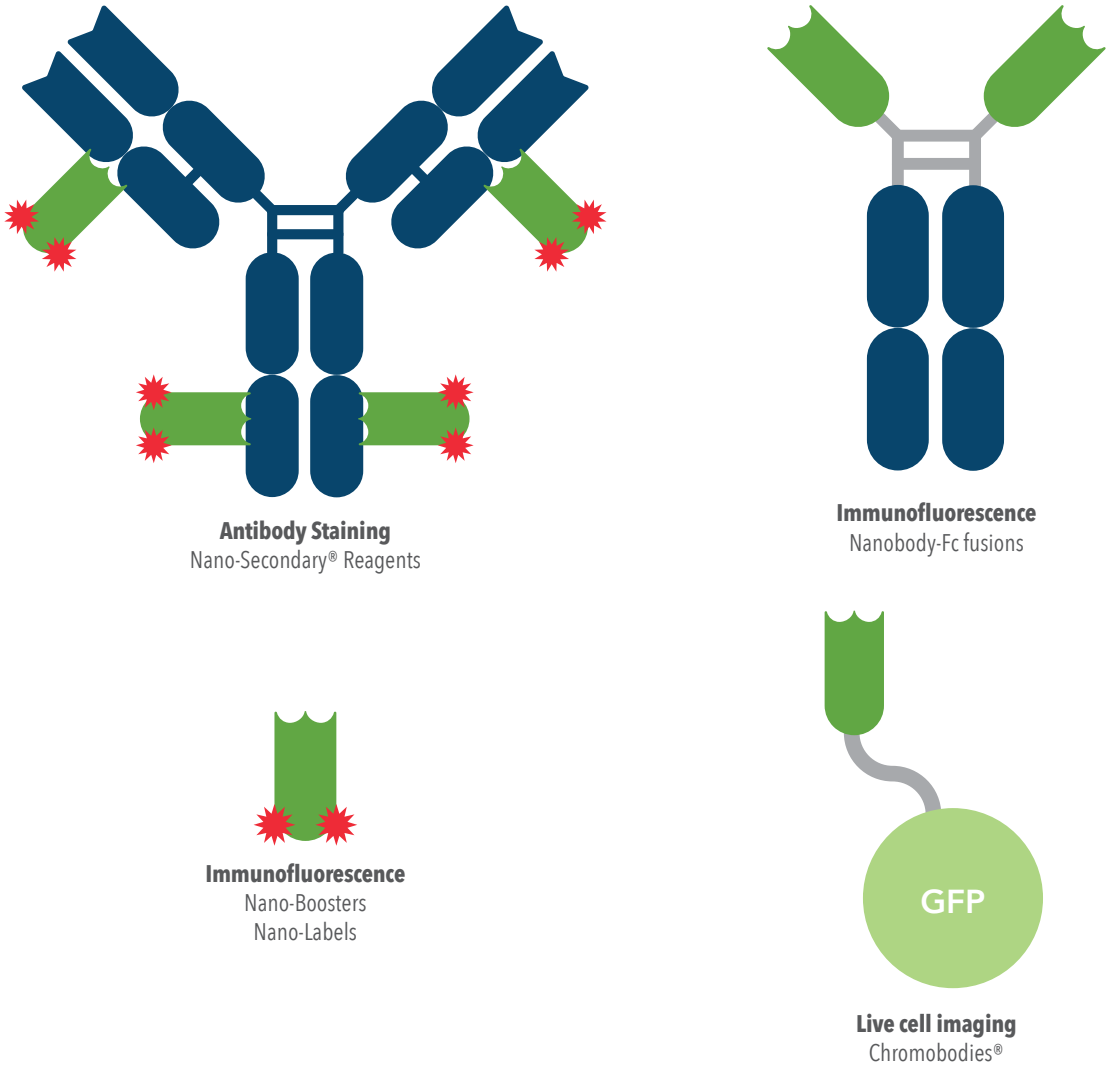
Conventional antibody

Nanobody/ VHH



Alpaca heavy-chain-only antibody

BETTER IMAGES WITH NANOBODIES



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

Nanobodies are the smallest known antibodies. Due to their small size, high specificity, low background, and consistently high quality, they enable better performance during immunofluorescence experiments.

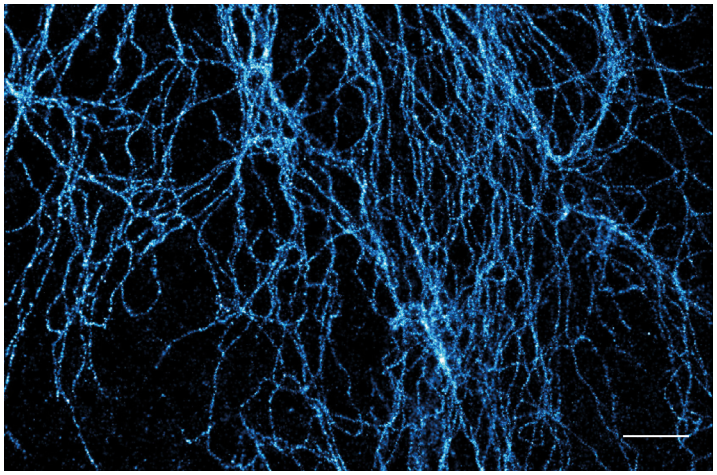
ChromoTek Nanobody-based research reagents for imaging consist of

- **Nano-Secondary® Reagents:** Nanobodies conjugated to fluorescent dyes bound 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, and Nano-Labels bind to non-fluorescent proteins or peptide tags.
- **Nanobody-Fc fusions:** Nanobodies fused to Fc domains.

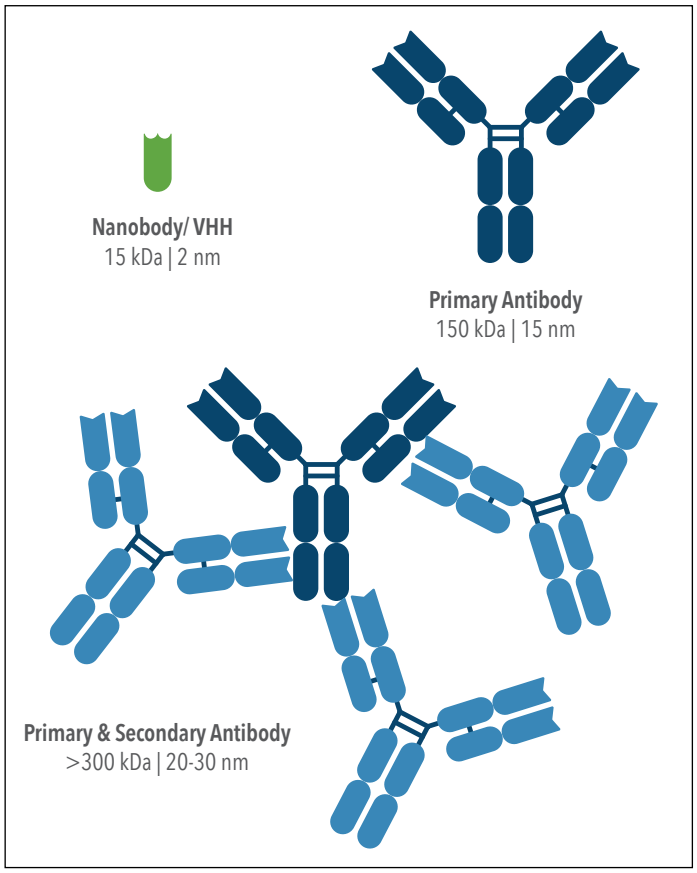
THE CHROMOTEK NANOBODY ADVANTAGES IN IMAGING

Small size of Nanobodies

Nanobodies are single-domain antibody fragments with a molecular weight of only 15 kDa and a size of 2 nm. Their small size is ideal for immunofluorescence assays.



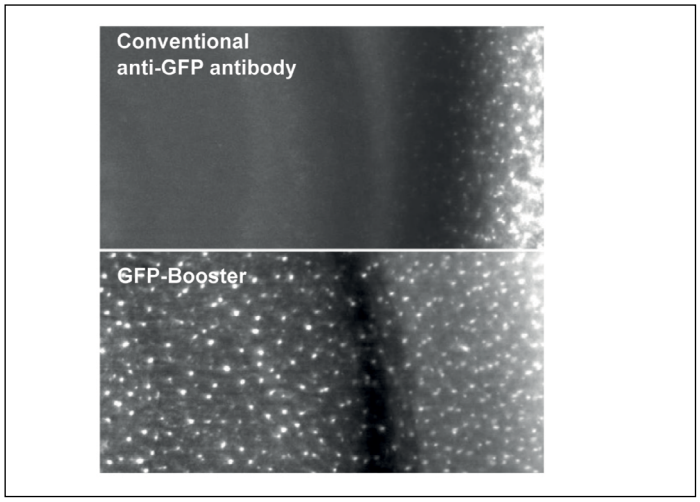
▲ Vimentin in HeLa cells. Cells were stained with monoclonal mouse IgG1 anti-Vimentin antibody and Nano-Secondary® alpaca anti-mouse IgG1, recombinant VHH, Alexa Fluor® 488 [CTK0103, CTK0104]. Courtesy of Dr. Leila Nahidiazar, Dr. Jop Kind, and Prof. Kees Jalink, from the Hubrecht Institute and The Netherlands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



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

Better tissue penetration

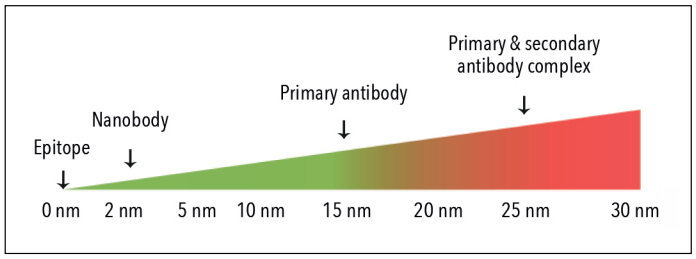
Nanobodies enable better and faster tissue penetration in crowded cellular environments and tissues as they are 10 times smaller than conventional IgG antibodies. The penetration rate directly influences image quality and incubation time, especially in immunostainings of tissues, organs, or whole animals.



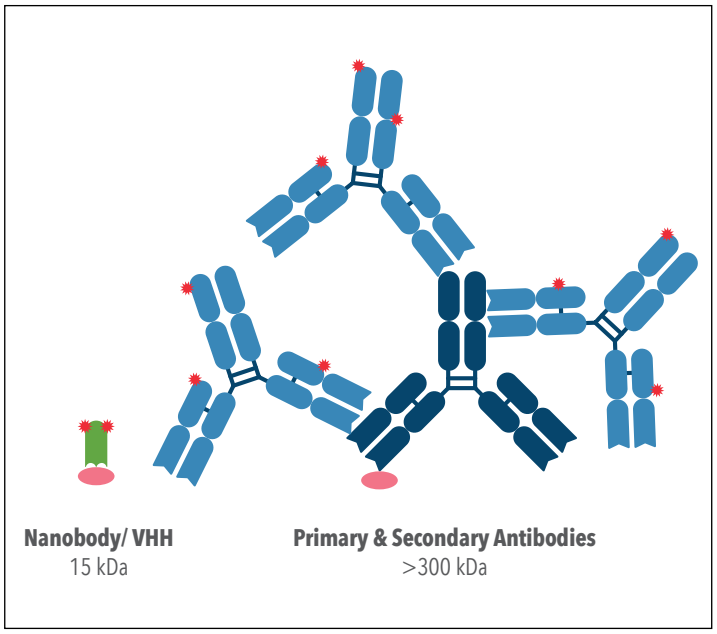
▶ Comparison of conventional anti-GFP antibody and GFP-Booster shows the superior tissue penetration rate of GFP-Booster. Fluorescent images of transgenic mouse tissue expressing Cx3Cr1-EGFP. EGFP signal was enhanced either with conventional anti-GFP antibody or with GFP-Booster.

Minimal label displacement

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



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

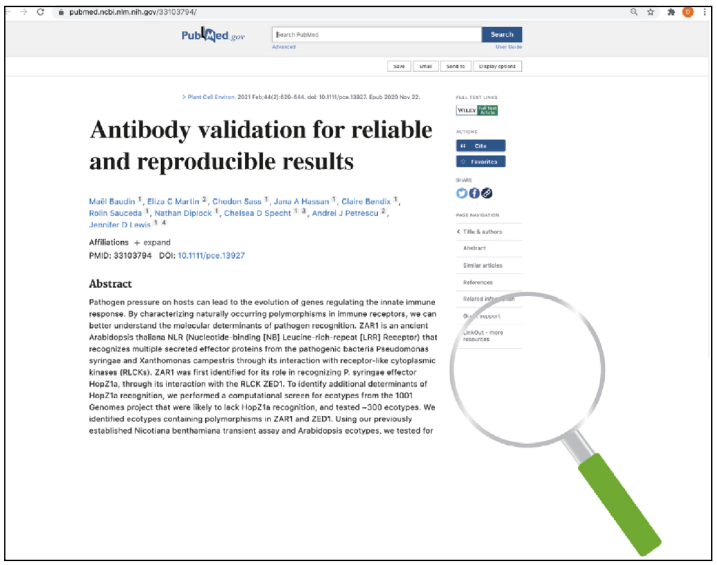


▲ Epitope to label displacement: Schematic comparison of a Nanobody (green) conjugated to fluorescent dyes (red) vs. a conventional primary antibody (dark blue) and secondary antibodies (light blue) conjugated to fluorescent dyes. Epitope is shown in pink.

Reproducibility and validation

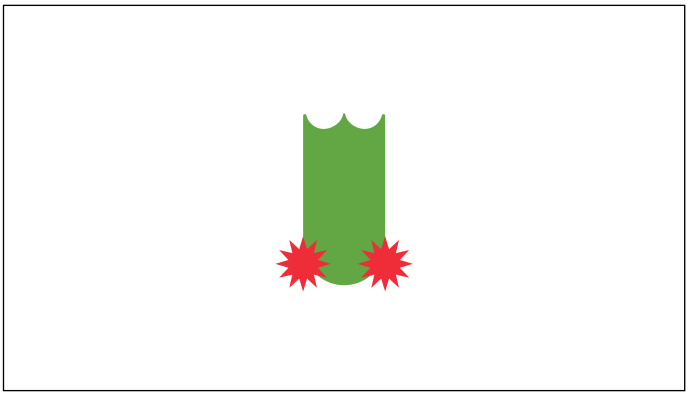
All ChromoTek Nanobodies including Nano-Secondary® Reagents, Nano-Boosters, Nano-Labels, Chromobodies®, and Nanobody-Fc fusions are thoroughly characterized and tested for high specificity, high affinity, and low background. ChromoTek Nanobodies are validated by genetic approaches. They are tested in 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.



Fluorescent conjugation

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

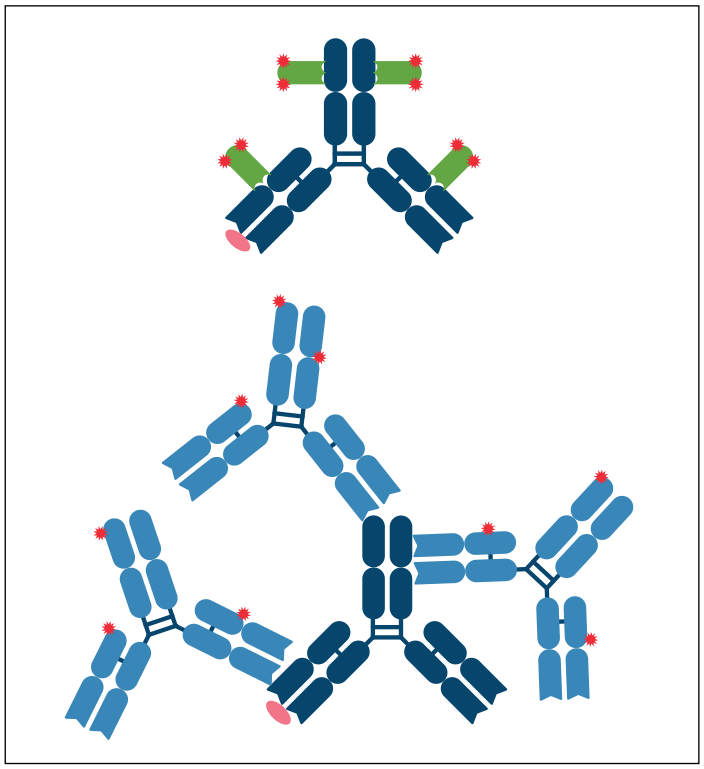


▲ Nanobody (green) conjugated with two fluorescent dyes (red).

NANO-SECONDARY® REAGENTS:

The next level of secondary antibodies

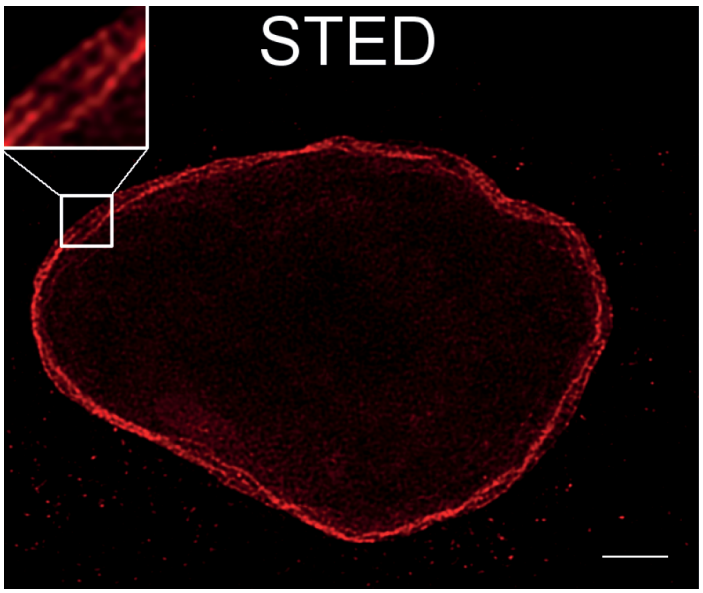
Nano-Secondary® Reagents are a novel class of secondary antibodies for higher resolution, cleaner images, and faster immunostaining. Nano-Secondary® Reagents are monovalent and consist of Nanobodies that bind to primary antibodies with high affinity in a species- and isotype-specific manner. Nano-Secondary®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



▲ Primary antibody (dark blue) & Nano-Secondary® Reagent (green) complex. Epitope is shown in pink and conjugated fluorescent dyes are shown in red. Complex of primary (dark blue) and polyclonal secondary antibodies (light blue). Epitope is shown in pink and conjugated fluorescent dyes are shown in red.

Super-resolution microscopy

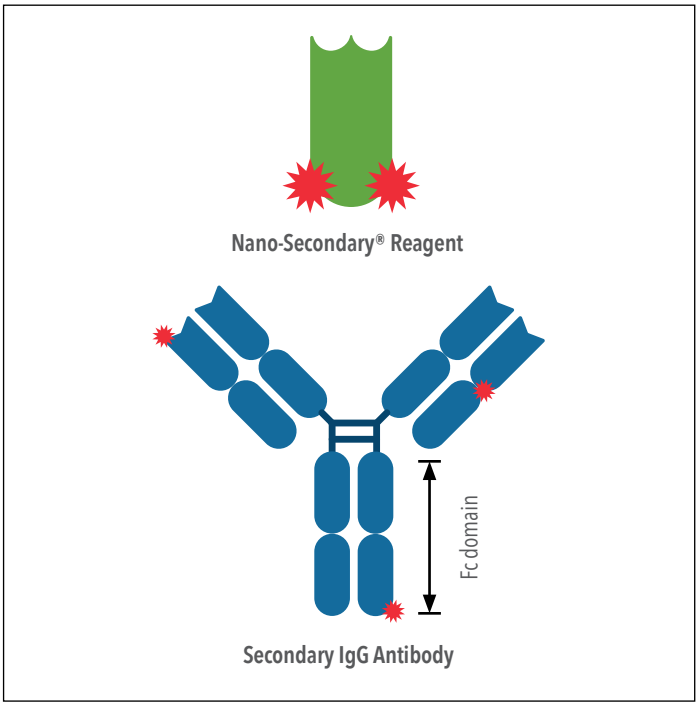
Due to their small size, Nano-Secondary® Reagents are excellent tools for super-resolution microscopy, e.g., STED, STORM. Using Nano-Secondary® Reagents it is possible to obtain a high precision staining and increase the resolution of images. Nano-Secondary® Reagents are offered with different bright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



▶ HeLa cells were immunostained with rabbit IgG anti-Lamin B1 antibodies and Nano-Secondary® alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor® 568 [CTK0101, CTK0102]. 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.

Low background

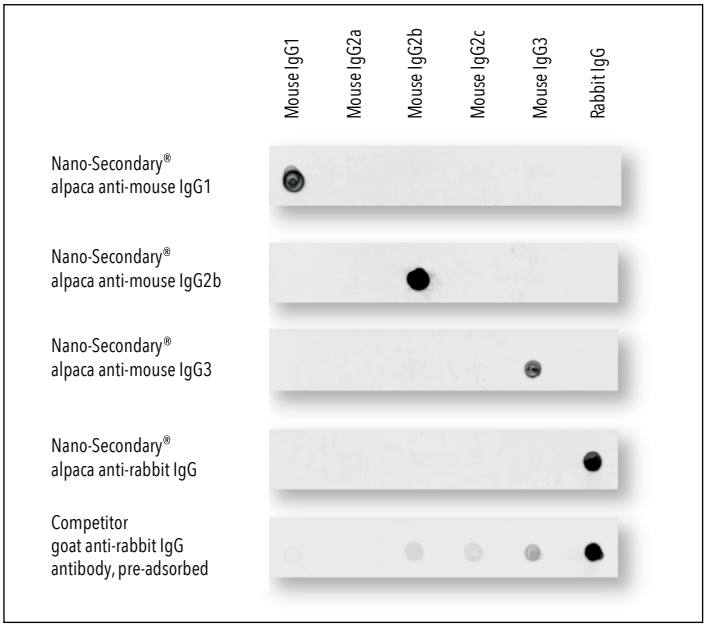
Nano-Secondary® Reagents show very low background staining. Only Nano-Secondary® Reagents with high specificity, high sensitivity, and low background are selected during development to minimize off-target effects. Accordingly, they detect isotype-specific IgGs and do not nonspecifically bind to other cellular components. Since Nano-Secondary® Reagents do not contain an Fc domain, they cannot be bound to endogenous Fc-receptors. Nano-Secondary® Reagents are ideal choices because of their low background and excellent signal-to-noise ratio.



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

No cross-reactivity

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



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

One-step incubation to save time

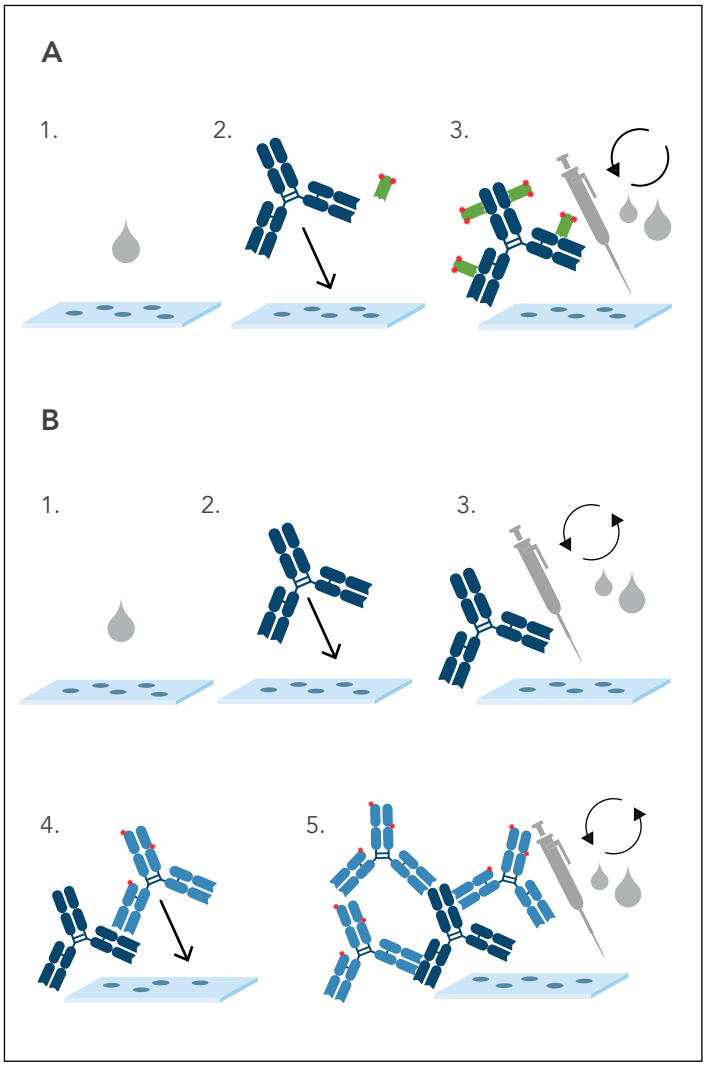
ChromoTek Nano-Secondary® Reagents are monovalent and enable one-step immunostaining: the simultaneous incubation of the sample with the primary antibody and Nano-Secondary® Reagent. This saves incubation time, reduces washing steps and hands-on time, and results in gentle incubation of the sample. One-Step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

A. One-step incubation with Proteintech primary antibodies and ChromoTek Nano-Secondary® Reagents

1. Fix, permeabilize and block I 2. Incubation with conventional primary and Nano-Secondary® Reagents I 3. Wash I 4. Detect

B. Traditional workflow with conventional primary and secondary antibodies.

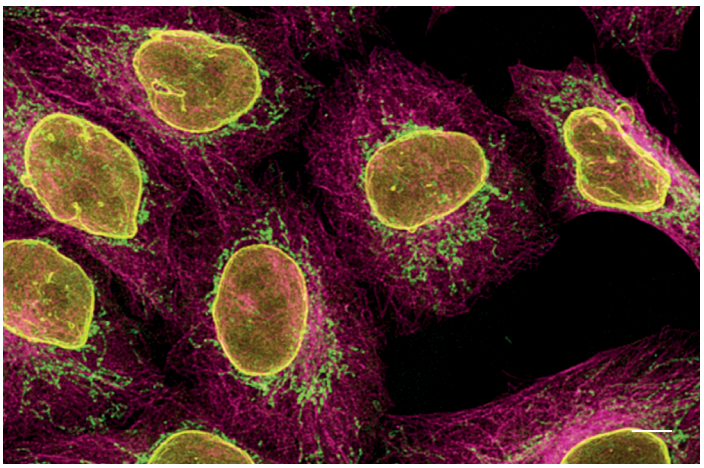
1. Fix, permeabilize and block I 2. Incubate with primary antibody I 3. Wash I 4. Incubate secondary antibody I 5. Wash I 6. Detect



▲ Workflow comparison of a time-saving using primary antibodies and Nano-Secondary® Reagents vs. immunostaining using primary and conventional secondary antibodies.

Multiplexing

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

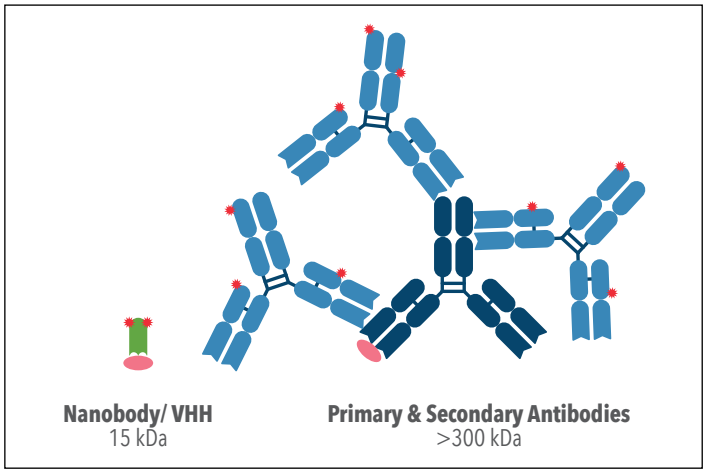


▲ Multiplexing of HeLa cells with Nano-Secondary® Reagents. Yellow: rabbit IgG anti-Lamin + Nano-Secondary® alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor® 568 [CTK0101, CTK0102], green: mouse IgG1 anti-COX4 + Nano-Secondary® alpaca anti-mouse IgG1, recombinant VHH, Alexa Fluor® 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.

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 widefield, 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®).



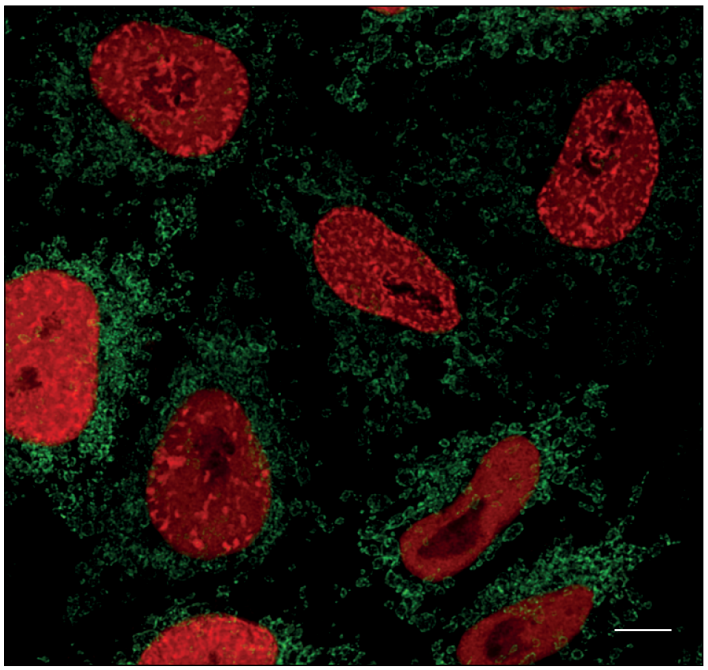
▲ Epitope to label displacement: Schematic comparison of a Nanobody (green) conjugated to fluorescent dyes (red) vs. a conventional primary antibody (dark blue) and secondary antibodies (light blue) conjugated to fluorescent dyes. Epitope is shown in pink.

Enhancing, stabilizing, and reactivating the signal of fluorescent proteins

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

- The signal intensities of fixed samples from cells expressing fluorescent protein fusions at physiological expression levels are usually very low.
- Neither the photostability nor quantum efficiency of fluorescent proteins 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 EvdU-Click-iT™ treatment, or heat denaturation for FiSH lead to disruption of the fluorescent protein signal.

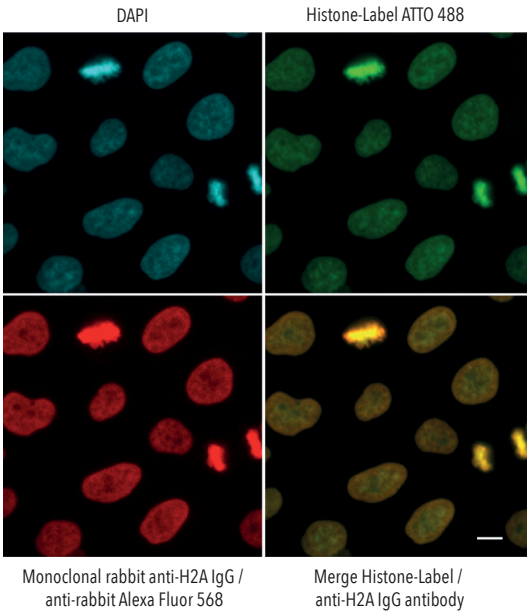
GFP-Booster and RFP-Booster stabilize, enhance, and reactivate the fluorescent signals of fluorescent proteins such as GFP, RFP, and mCherry in immunofluorescence. They are monovalent and are offered labeled with various Alexa Fluor® dyes or ATTO dyes.



▲ HeLa cells transiently transfected with PCNA-mRFP and Tom 70-EGFP were subjected to 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.

Direct immunostaining of endogenous proteins

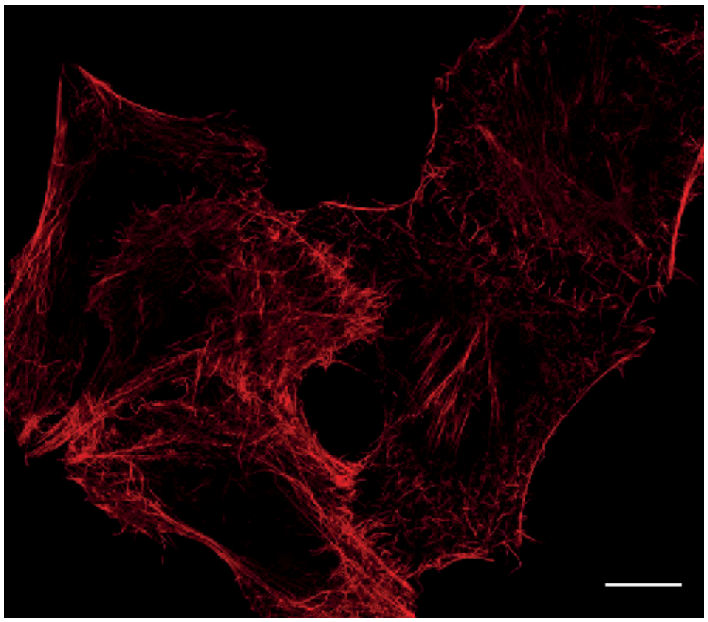
Nano-Labels precisely stain proteins in their cellular environment. Due to their minimal epitope-label displacement and superior accessibility to epitopes in crowded cellular/organelle environments, Nano-Labels enable high resolution immunofluorescence and microscopy. The Vimentin-Label specifically 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 the nucleus and provides more precise staining (see legend).



▲ 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.

Peptide tag-specific Nanobody for immunofluorescence: Spot-Label®

The Spot-Label® is the first Nanobody to be used in super-resolution 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.

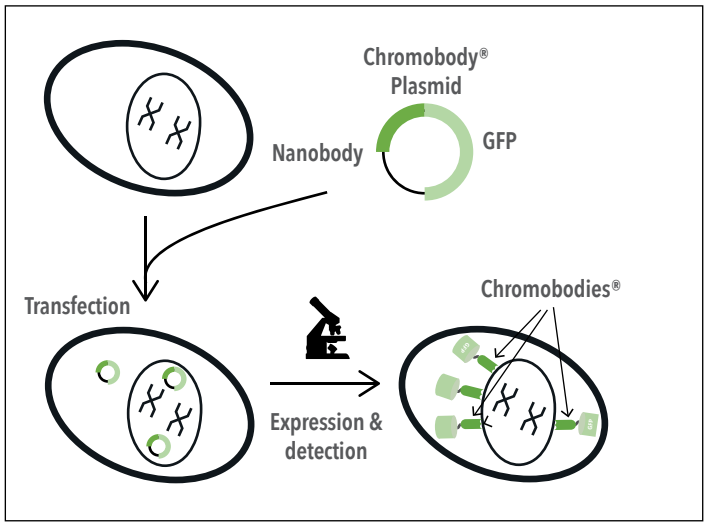


▲ STED super-resolution imaging of Spot-tagged Actin-Chromobody® with Spot-Label® ATTO 594. Gated STED images were acquired with a Leica TCS SP8 STED 3X microscope with pulsed White Light Laser excitation at 590 nm and pulsed depletion with a 775 nm laser. Objective: 100x Oil STED White, NA: 1.4. Pixel 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

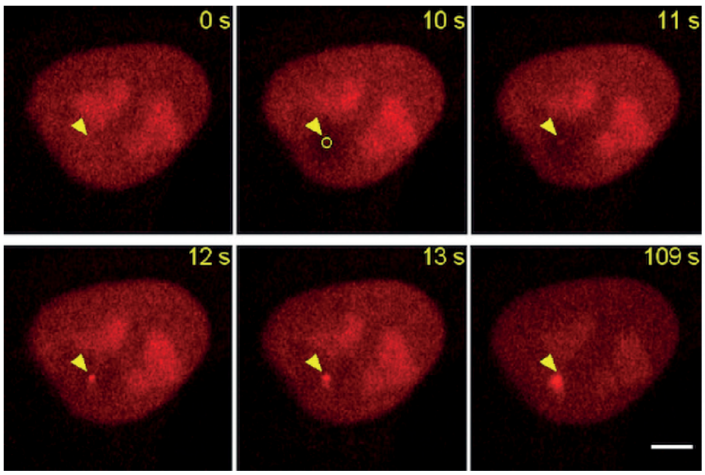
Chromobodies® are small intracellular functional antibodies that work as fluorescent live-cell nanoprobes. They are optimized for real-time and live-cell imaging of endogenous proteins. Chromobodies® are Nanobodies that are genetically fused to a fluorescent protein such as GFP, and are also available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



▲ 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.

Real-time imaging

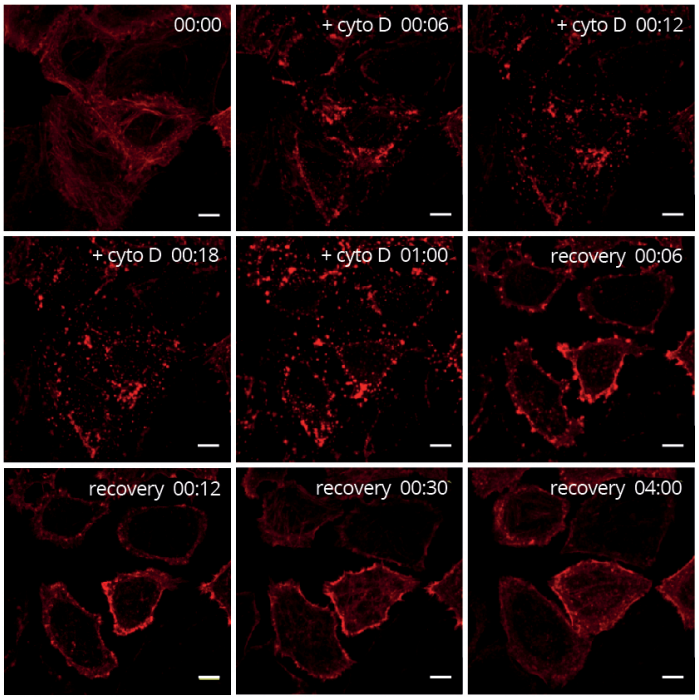
Upon transfection, the Chromobody® protein is intracellularly expressed, binds to the endogenous protein, and brings the fluorescent protein to the target structure. Chromobodies® can be applied to monitor protein dynamics or visualize biomarkers in secondary screens in real-time in live cells.



▲ 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 triangle shows the location of the microirradiation. Time-lapse analysis shows the recruitment of PARP1 to DNA damage. Scale bar, 5 µm.

Minimal interference with target function

Chromobodies® non-invasively label endogenous proteins without interfering with protein functions. 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.

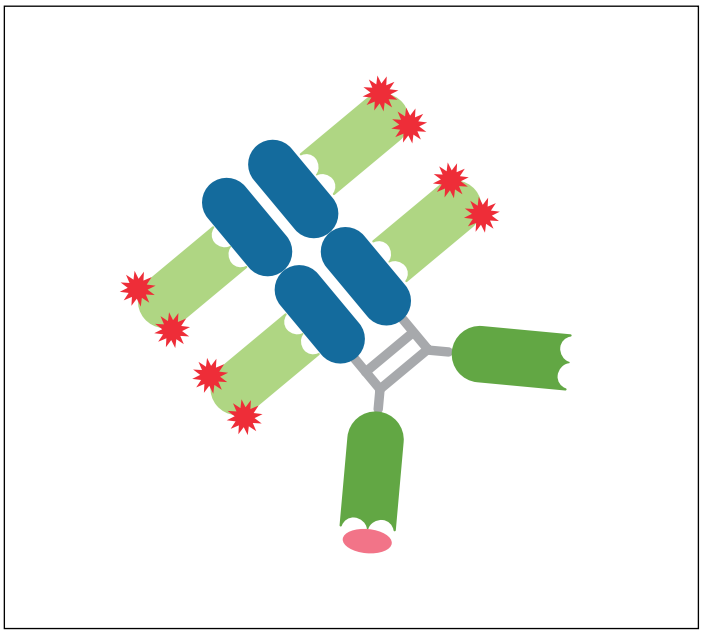


▲ The time series reveals the reorganization of actin after treatment with Cytochalasin D: HeLa cells were subjected to confocal imaging upon treatment with 2 µM of Cytochalasin D for 1 hr and recovery for 4 hr. Actin-Chromobody® enables monitoring of Actin dynamics in real time in living cells. Scale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

NANOBODY-FC FUSIONS:

The Nanobody advantage combined with traditional IgG antibodies

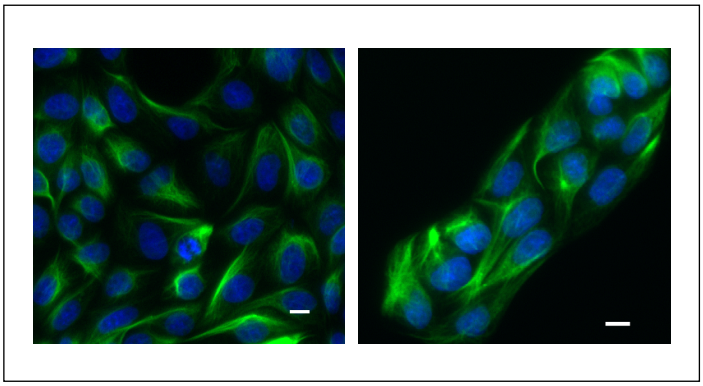
Nanobodies can also be fused to Fc domains to generate chimeric heavy-chain antibodies. The Fc domain fusion format combines the advantages of Nanobodies with those of traditional antibodies. Nanobody-Fc fusions are bivalent, resulting in even higher affinities due to an avidity effect, can bind unique 3-dimensional conformations of epitopes not recognized by traditional antibodies, and can be detected and captured using tools from the broad range of antibody reagents like secondary antibodies and Nano-Secondary® Reagents. 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-Secondary® Reagents (light green). Epitope is shown in pink and conjugated fluorescent dyes are shown in red. Nanobody-Fc fusions can also be detected using traditional secondary antibodies.

Increased flexibility

Nanobody-Fc fusions are available with rabbit IgG or mouse IgG1 Fc domains. Different Nanobody-Fc fusion versions consist of the same Nanobody and show the same high specificity but offer increased flexibility. By offering a selection of Fc domains, different secondary antibodies can be used for detection, which enables Nanobody-Fc fusions to fit perfectly into a multiplex panel.



▲ MDCK cells were immunostained with Vimentin recombinant antibody, Nanobody-rabbit IgG Fc fusion (left) or Vimentin recombinant antibody, Nanobody-mouse IgG1 Fc fusion (right). Nanobody-Fc fusions were detected with Nano-Secondary ® alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor® 647 [CTK0101,CTK0102] and Nano-Secondary® alpaca anti-mouse IgG1, recombinant VHH, Alexa Fluor® 647 [CTK0103, CTK0104]. DAPI in blue. Scale bar, 10 µm.

PRODUCT OVERVIEW:

Nanobody-based Reagents for Imaging

Nano-Secondary® Reagents	Nano-Boosters & Nano-Labels	Chromobodies®	Nanobody-Fc Fusions
Nano-Secondary® alpaca anti-mouse IgG1	GFP-Booster	Actin-Chromobody®	GFP recombinant antibody, VHH-mouse IgG1 Fc fusion
Nano-Secondary® alpaca anti-mouse IgG2a	RFP-Booster	Nuclear Actin-Chromobody®	GFP recombinant antibody, VHH-rabbit IgG Fc fusion
Nano-Secondary® alpaca anti-mouse IgG2b	Spot-Label®	Cell Cycle-Chromobody®	mNeonGreen recombinant antibody, VHH-mouse IgG1 Fc fusion
Nano-Secondary® alpaca anti-mouse IgG3	Histone-Label	Dnmt1-Chromobody®	mNeonGreen recombinant antibody, VHH-rabbit IgG Fc fusion
Nano-Secondary® alpaca anti-mouse Ig kappa light chain	Vimentin-Label	Histone-Chromobody®	TurboGFP recombinant antibody, VHH-mouse IgG1 Fc fusion
Nano-Secondary® alpaca anti-rabbit IgG		Lamin-Chromobody®	TurboGFP recombinant antibody, VHH-rabbit IgG Fc fusion
Nano-Secondary® alpaca anti-human IgG		PARP1-Chromobody®	Vimentin recombinant antibody, VHH-mouse IgG1 Fc fusion
		Vimentin-Chromobody®	Vimentin recombinant antibody, VHH-rabbit IgG Fc fusion

proteintech®

CONTACT US

Proteintech Germany GmbH
ChromoTek GmbH

Am Klopferspitz 19
82152 Planegg-Martinsried
Germany

P +49 89 124 148 850
F +49 89 124 148 811
E germany@ptglab.com

Proteintech Europe

4th Floor, 196 Deansgate
Manchester
M3 3WF. United Kingdom

P +44 161 839 3007
F +44 161 241 3103
E europe@ptglab.com

Proteintech Group, Inc.
(HQ in North America)

5500 Pearl Street, Ste 400
Rosemont, IL 60018, USA

P +1 (888) 478-4522 (toll free in USA)
F +1 (773) 598-4321
+1 (847) 928-2189
E proteintech@ptglab.com

ptglab.com