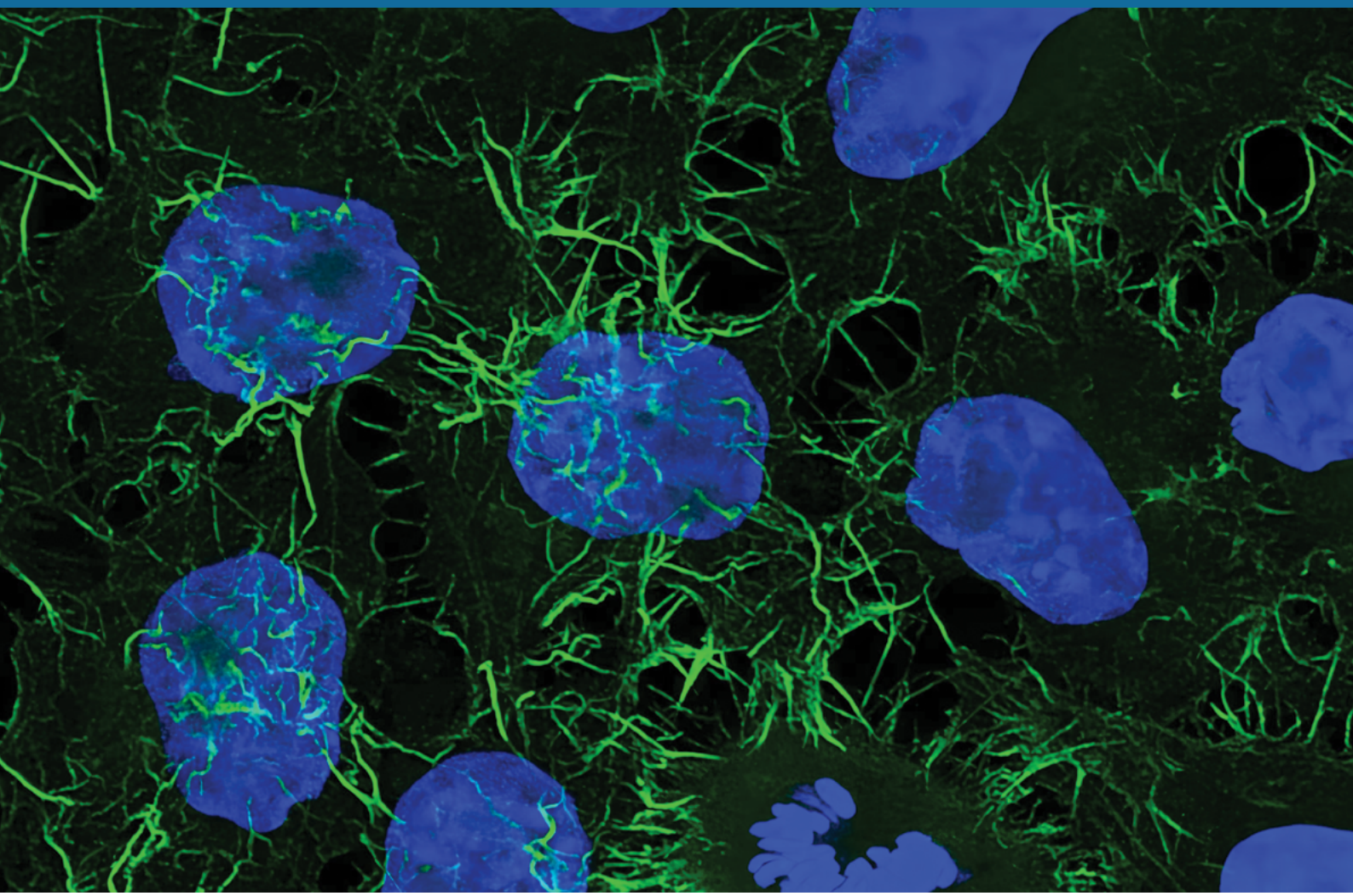
proteintech®

chromotek®

Nanobody-based Reagents



Find it at **eu.fishersci.com**

fisher scientific
part of Thermo Fisher Scientific

TABLE OF CONTENTS

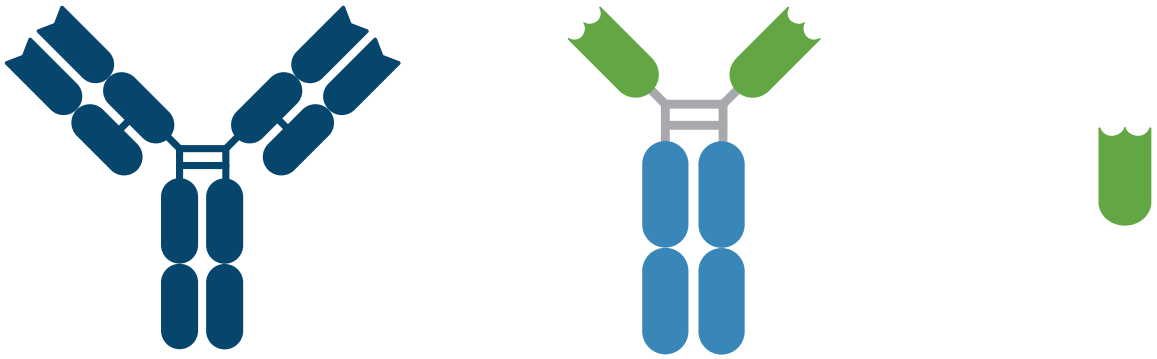
ON THE COVER: Immunofluorescence analysis of HeLa cells stained with mouse IgG2b anti-tubulin beta antibody and Nano-Secondary® alpaca anti-mouse IgG2b, recombinant VHH, CoraLite® Plus 488 (smsG2bCL488-1, green). Nuclei were stained with DAPI (blue). Images were recorded at the Core Facility Bioimaging at the Biomedical Center, LMU Munich.

ChromoTek®
NANOBODY-BASED REAGENTS

Proteintech's range of ChromoTek Nanobody-based reagents offers premium research tools that provide a higher level of performance compared to conventional IgG antibodies in applications such as immunoprecipitation, immunofluorescence, live-cell imaging, biosensor assays, and protein purification.

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.



Conventional antibody | **Alpaca heavy-chain-only antibody** | **Nanobody/ VHH**

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

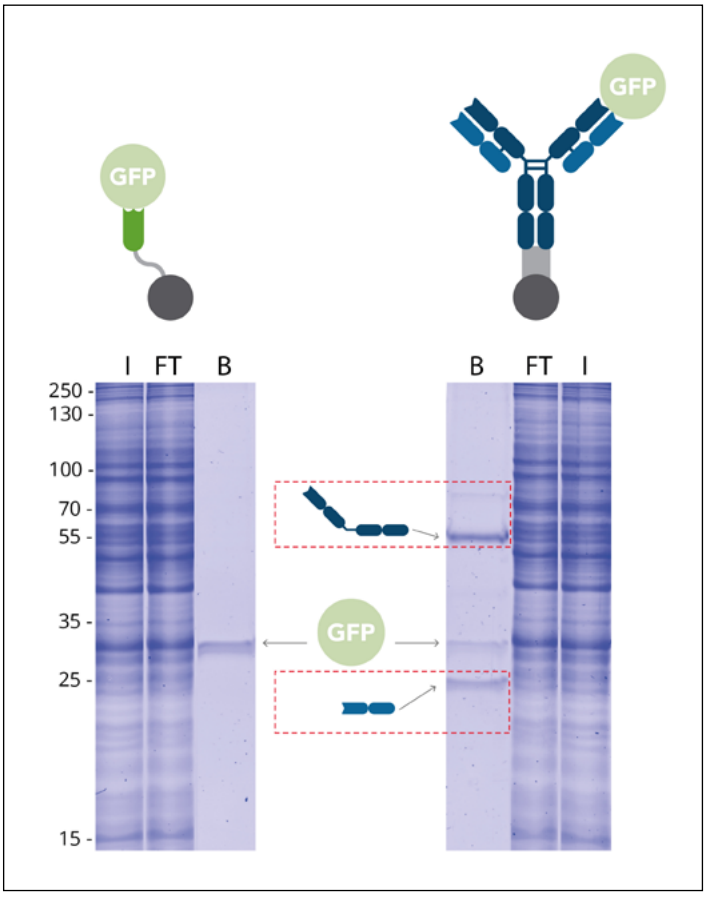
NANO-TRAPS:

Ready-to-use affinity beads for fast and efficient immunoprecipitation

ChromoTek Nano-Traps are the benchmark in immunoprecipitation and allow fast and reliable one-step pulldowns of low expressed proteins. They consist of Nanobodies coupled to beads and are ready to use in the following applications: immunoprecipitation (IP), Co-IP, Co-IP/-mass spectrometry, on-bead assays, ChIP/RIP analysis, and split-fluorescent protein assays.

Benefits:

- No heavy & light antibody chains
- Stringent washing conditions
- Low host cell protein background
- Very efficient pulldown, no protein in flow-through
- High affinity to bind proteins expressed at low levels
- Short incubation (30-60 min)
- Recombinantly expressed and validated



▲ Immunoprecipitation of GFP by GFP-Trap® compared with a conventional anti-GFP antibody coupled to Protein A/G beads analyzed by SDS-PAGE. I: Input, FT: Flow-Through, B: Bound.

Scan to Discover More

**Note that the DYKDDDDK Fab-Trap® contains a Fab Fragment*

NANO-SECONDARY® REAGENTS:

Immunostaining reagents for higher-resolution imaging

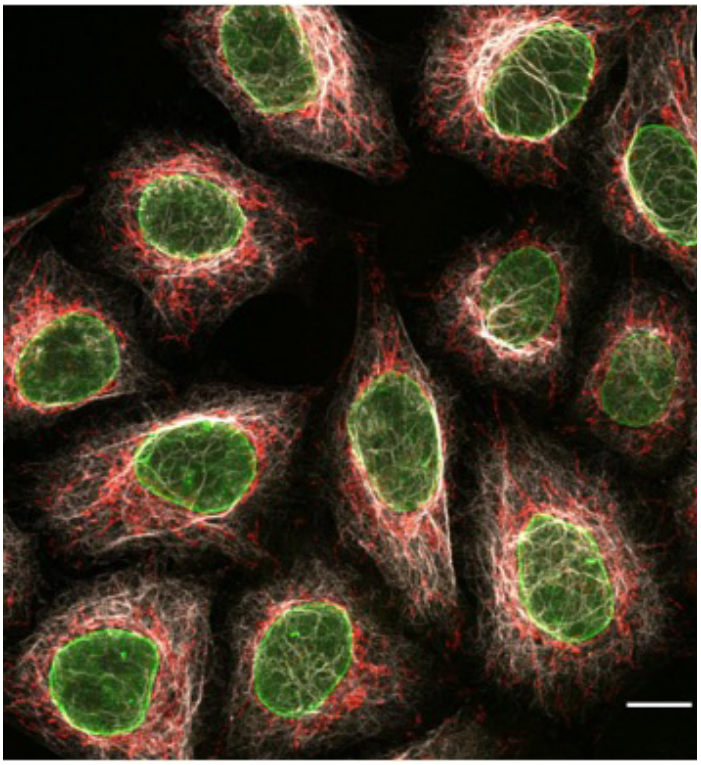
Nano-Secondary® reagents are a novel class of recombinant secondary antibodies designed to provide higher resolution, lower background, and cleaner images. Nano-Secondary reagents have recombinantly generated Nanobodies/ VHHs conjugated to Alexa Fluor® or CoraLite® Plus fluorescent dyes and they bind to primary antibodies with high affinity in a species and subtype specific manner.

Benefits:

- Higher resolution
- Better tissue penetration
- Low background
- No cross-reactivity and high subclass specificity
- Same species multiplexing
- High lot-to-lot consistency

Applications:

- Immunofluorescence
- Super-resolution microscopy
- Western blotting
- Flow cytometry



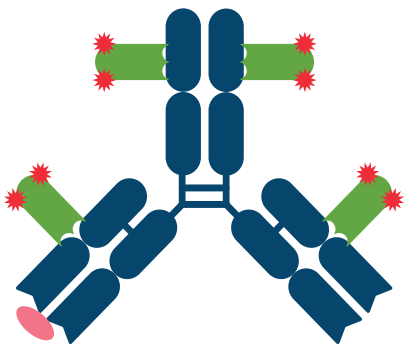
▲ Multiplexed immunostaining of HeLa cells with 3 subclass-specific alpaca anti-mouse Nano-Secondary reagents. Gray: Mouse IgG1 anti-Vimentin + alpaca anti-mouse IgG1 VHH Alexa Fluor® 647. Green: Mouse IgG2b anti-Lamin + alpaca anti-mouse IgG2b VHH Alexa Fluor® 488. Red: Mouse IgG3 anti-MOT + alpaca anti-mouse IgG3 VHH Alexa Fluor® 568.

Specificities:

- Human IgG
- Mouse IgG1, IgG2a, IgG2b, IgG3
- Rabbit IgG

Conjugates:

- Alexa Fluor® 488, 568, 647
- CoraLite® Plus 488, 555, 647, 750



◀ Primary antibody (dark blue) and Nano-Secondary® Reagents (green) complex. Epitope is shown in pink and conjugated fluorescent dyes are shown in red.

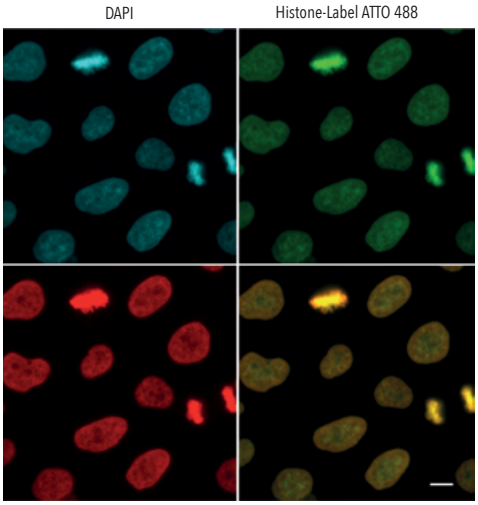
Scan to Discover More

NANO-BOOSTERS AND NANO-LABELS:
Fluorescent probes for immunofluorescence

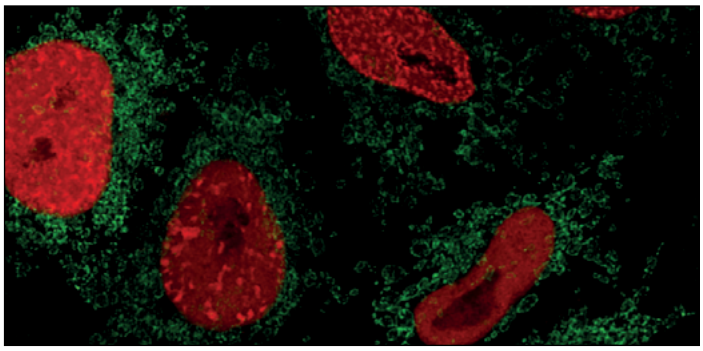
Nano-Boosters and Nano-Labels are pre-conjugated fluorescent probes that enable higher image quality in widefield, confocal, and super-resolution microscopy. Nano-Boosters stabilize, enhance, and reactivate the signal of fluorescent proteins (GFP- and RFP-Boosters), while Nano-Labels fluorescently label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

Benefits:

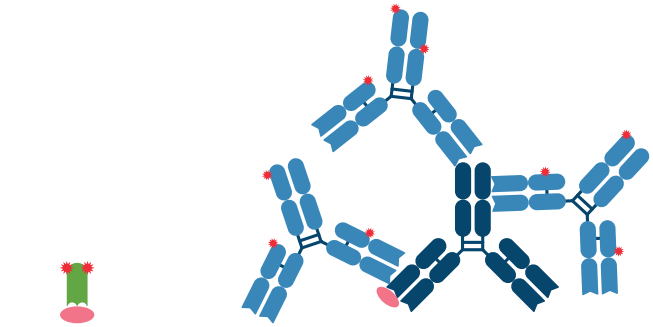
- Better tissue penetration
- Higher labeling density
- Minimal linkage error: Less than 2 nm epitope-label displacement
- GFP- and RFP-Boosters stabilize, retain, and enhance signals of fluorescent proteins
- Recombinantly expressed and validated
- Superior accessibility and labeling of epitopes in crowded cellular/organelle environments



▲ 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 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.



▲ 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).



▲ Displacement: Nanobody (green) conjugated to fluorescent dyes (red) vs. a conventional primary antibody (dark blue) and secondary antibodies (light blue) conjugated to fluorescent dyes. Epitope shown in pink.

Scan to Discover More

NANO-CAPTURELIGANDS®:
Site-directed immobilization of antibodies

ChromoTek Nano-CaptureLigands® are optimized for the site-directed and gentle immobilization of antibodies. They specifically capture non-biotinylated immunoglobulins to streptavidin/avidin. Nano-CaptureLigands® are biotinylated VHHs/ Nanobodies.

Benefits:

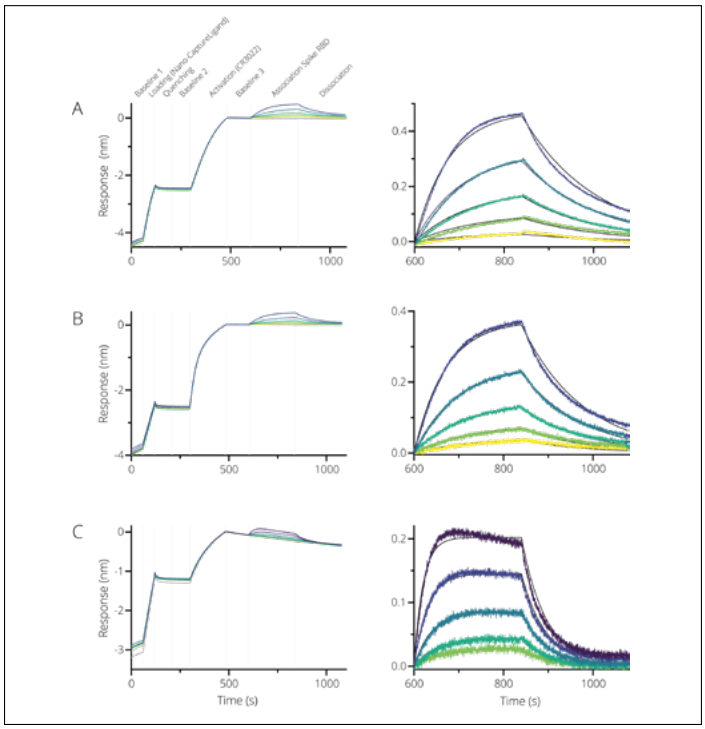
- Gentle and site-directed immobilization of antibodies
- Works with crude samples
- Multiple regeneration cycles
- No antibody biotinylation required

Applications:

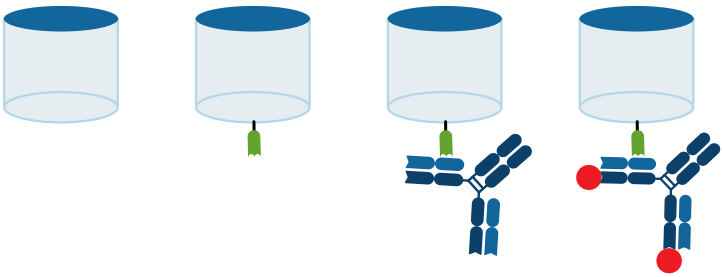
- BLI
- SPR
- ELISA

Targets:

- Human Ig, lambda
- Human IgE
- Human IgG
- Mouse IgE
- Mouse IgG1
- Mouse IgG2a
- Mouse IgG2b
- Rabbit IgG



▲ BLI kinetics of the binding of human and mouse antibody CR3022 to SARS-CoV2 Spike RBD. Human and mouse antibody CR3022 was captured on a streptavidin biosensor using biotinylated Nano-CaptureLigands® and assayed with different concentrations of SARS-CoV2 Spike RBD. A: Human IgG1 antibody CR3022 was captured by Nano-CaptureLigand® human IgG/rabbit IgG, Fc-specific VHH, biotinylated. B: A chimeric mouse IgG2b antibody CR3022 was captured by Nano-CaptureLigand® mouse IgG2b, Fc-specific VHH, biotinylated. C: A chimeric mouse IgG2b antibody CR3022 was captured by Nano-CaptureLigand® mouse IgG, Fab-kappa-LC-specific VHH, biotinylated.



▲ Immobilization of the Nano-CaptureLigand® (green) on Biosensor (white) for biolayer interferometry followed by capture of antibody (orange). The setup is ready for characterization of antibodies' binding kinetics and affinity to substrate (red), antibody discovery, and screening in crude liquids such as hybridoma supernatant, serum, and plasma.

Scan to Discover More

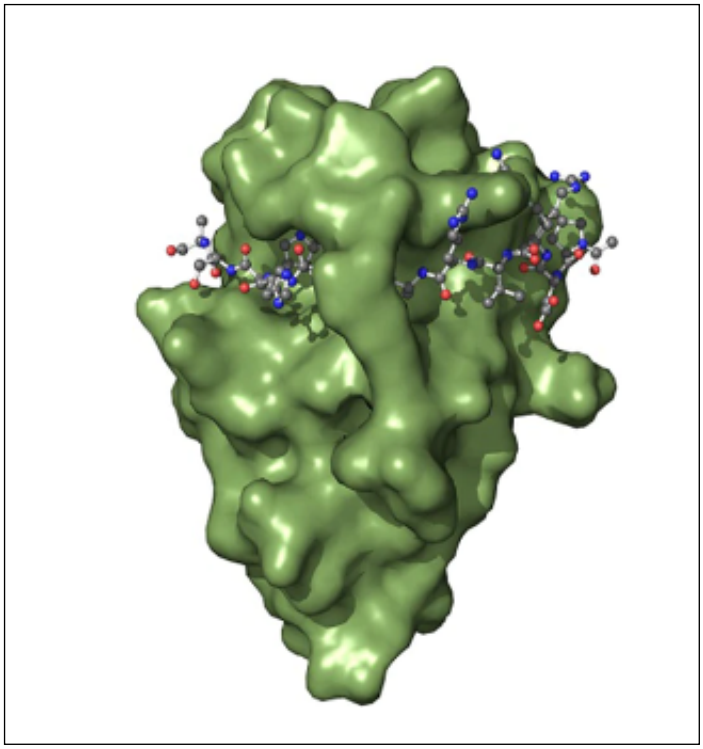
SPOT-TAG®:
A novel capture & detection system

ChromoTek's proprietary Spot-Tag® system consists of the first Nanobody for universal capture & detection applications. The Spot-Tag® is an inert 12-amino-acid peptide-tag (PDRVRAVSHWSS) with Spot-Nanobodies that specifically bind to Spot-tagged proteins with high affinity.

Tools & Applications:

- Spot-Trap® for immunoprecipitation/Co-IP
- Spot-Trap®/iST Spot-Trap® Kit for mass spectrometry
- Spot-Cap® for protein purification
- Spot-Label® for immunofluorescence
- Spot-Tag® antibody [28a5] for western blotting

Scan to Discover More



▲ Interaction of Spot-Nanobody (green) with Spot Peptide. The soluble accessible surface of the Spot VHH is shown in green.

NANO-CAPS®:
Optimized for protein purification

Nano-Caps® are premium affinity resins optimized for effective and economic one-step protein purification. They consist of Nanobodies/VHHs conjugated to agarose beads.

Benefits:

- Fast one-step protein purification at 4°C
- High binding capacity
- Efficient peptide elution
- Frequent regeneration for multiple reuse
- High specificity

Scan to Discover More

CHROMOBODIES®:
Intracellular Nanobodies for live-cell analysis

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

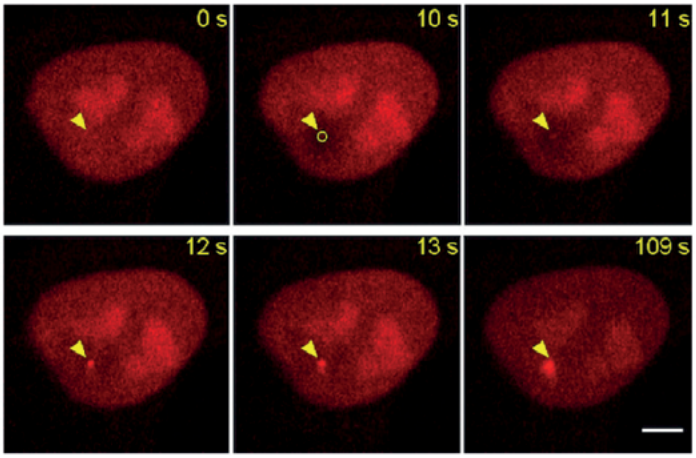
Scan to Discover More

Benefits:

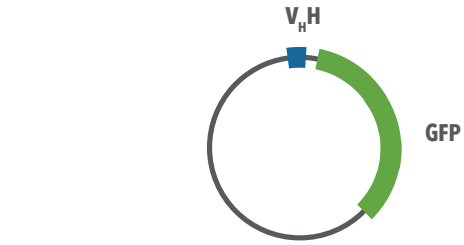
- Fast no-wash assay
- No overexpression of the protein of interest
- No cytotoxicity and artificial effects

Tools & Applications:

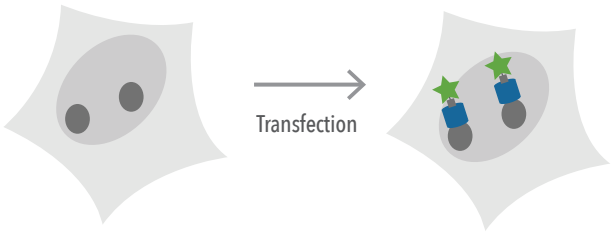
- Live-cell imaging of endogenous proteins
- High-content analysis of cells, tissues, organs, organisms
- Investigation of small molecules in secondary screens
- Investigation of cellular processes
  - Cell cycle progression (Cell Cycle/PCNA Chromobody)
  - Epithelial-mesenchymal transition (EMT- Vimentin Chromobody)
  - DNA damage response (PARP1 Chromo body)



▲ 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.



Chromobody® plasmid



▲ Schematic of a cell expressing Chromobodies upon transfection with the Chromobody DNA plasmid. The Chromobody plasmid codes for a VHH/Nanobody that is genetically fused to GFP.

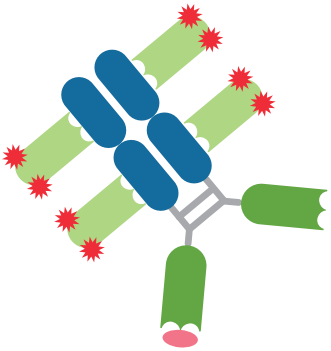
NANOBODY-FC FUSIONS
for increased flexibility

Nanobodies can be fused to Fc domains to generate chimeric heavy chain antibodies. This Fc-domain fusion format combines the advantages of Nanobodies with those of traditional antibodies: The VHH/Fc fusions are bivalent so they have even higher avidities and affinities than the parent Nanobodies, 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.

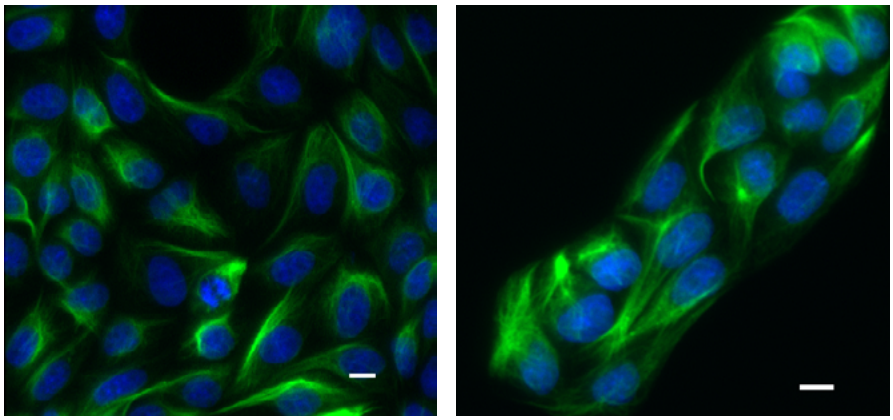
Scan to Discover More

Benefits:

- Recombinantly expressed for high lot-to-lot consistency and unlimited supply
- Bind unique epitopes that traditional antibodies do not recognize
- Compatible with Nano-Secondary® reagents



▲ 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.



◀ 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 [CT-K0101,CTK0102] and Nano-Secondary® alpaca anti-mouse IgG1, recombinant VHH, Alexa Fluor® 647 [CTK0103, CTK0104]. DAPI in blue. Scale bar, 10 µm.

NANOBODIES/VHHs:

Unconjugated primary single-domain antibodies

ChromoTek's Nanobodies/VHHs are well characterized and validated. Because the molecular weight of these single-domain antibodies is just 15 kDa, VHHs are only one-tenth the size of a conventional IgG antibody (150 kDa) or less than a third the size of a Fab fragment (50 kDa). Nanobodies are suitable for conjugation to dyes, biotin, beads, surfaces, etc., via NHS ester reaction.

Scan to Discover More

Fluorescent proteins

- GFP VHH
- GFP VHH, biotinylated
- TurboGFP VHH
- RFP VHH
- Halo VHH
- mNeonGreen VHH

Solubilization/Peptide tags

- GST VHH
- MBP VHH
- V5 VHH
- Myc VHH
- Spot VHH
- SNAP/CLIP-tag® VHH

Native targets

- N-term VHH
- p53 C-term VHH
- PARP1 VHH
- Vimentin VHH
- Histone VHH
- MK2 VHH
- Ubiquitin VHH

ANTIBODIES/IgGs:

Reliable detection of proteins, tags, and epigenetic markers

Proteintech's antibody portfolio spans over 13,000 targets including ChromoTek's IgG antibodies against commonly used protein tags. IgG antibodies use a different antigen recognition mode to Nanobodies. Nanobodies bind to conformational 3D epitopes, whereas conventional antibodies mostly bind linear epitopes found in denatured proteins, e.g., in western blots.

Targets

- GFP antibody [3H9]
- GFP antibody rabbit polyclonal [PABG1]
- GST antibody [6G9]
- HA antibody [7C9]
- Halo antibody [28A8]
- mNeonGreen antibody [32F6]
- Myc-tag antibody [9E1]
- RFP antibody [5F8]
- RFP antibody [6G6]
- mNeonGreen antibody [32F6]
- SNAP/CLIP-tag® antibody [6F9]
- V5-tag antibody [v5ab]

proteintech®

CONTACT US

Proteintech Europe

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

Proteintech Germany GmbH

ChromoTek GmbH
Fraunhoferstr. 1
82152 Planegg-Martinsried Germany

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

Proteintech China

E proteintech-CN@ptglab.com

Proteintech Singapore

P +65 9785 0861
E singapore@ptglab.com

ptglab.com

Distributed by Fisher Scientific. Contact us today:

Austria: fishersci.at **Belgium:** fishersci.be **Denmark:** fishersci.dk
Germany: fishersci.de **Ireland:** fishersci.ie **Italy:** fishersci.it
Finland: fishersci.fi **France:** fishersci.fr **Netherlands:** fishersci.nl
Norway: fishersci.no **Portugal:** fishersci.pt **Spain:** fishersci.es
Sweden: fishersci.se **Switzerland:** fishersci.ch **UK:** fishersci.co.uk

fisher scientific
part of Thermo Fisher Scientific