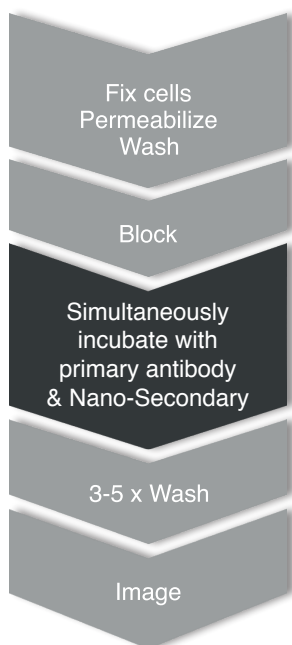# ChromoTek
new tools for better research

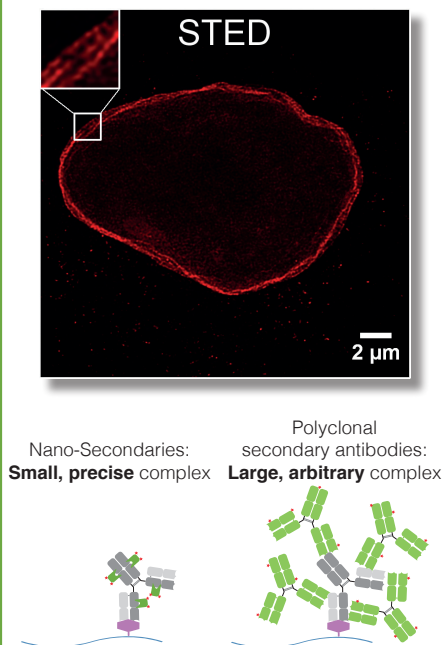# Nano-Secondaries: The next level of secondary antibodies

Nano-Secondaries are secondary antibodies. They are based on monoclonal recombinant V_HHs/ Nanobodies. Nano-Secondaries bind to primary antibodies with high affinity and specificity. Our Nano-Secondaries are conjugated to Alexa Fluor® dyes. Currently, Nano-Secondaries against rabbit and mouse IgG are available.

Faster Staining

Fix cells
Permeabilize
Wash

Block

Simultaneously incubate with primary antibody & Nano-Secondary
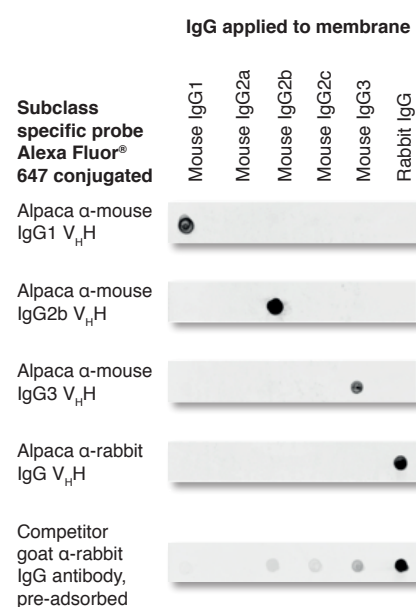
3-5 x Wash

Image

One-step immunostaining is the simultaneous incubation of primary antibody and Nano-Secondary.
This method reduces incubation and hands-on time. Simultaneous incubation also supports multiplexing, tissue penetration, and cell staining for flow cytometry.

Higher Resolution

STED

2 µm

Nano-Secondaries:
Small, precise complex

Polyclonal secondary antibodies:
Large, arbitrary complex

Nano-Secondaries are about 10 times smaller than conventional secondary antibodies. This small size enables better tissue penetration and decreases the distance between epitope and label. Nano-Secondaries are perfect probes for super-resolution microscopy, e.g. STED, STORM etc.
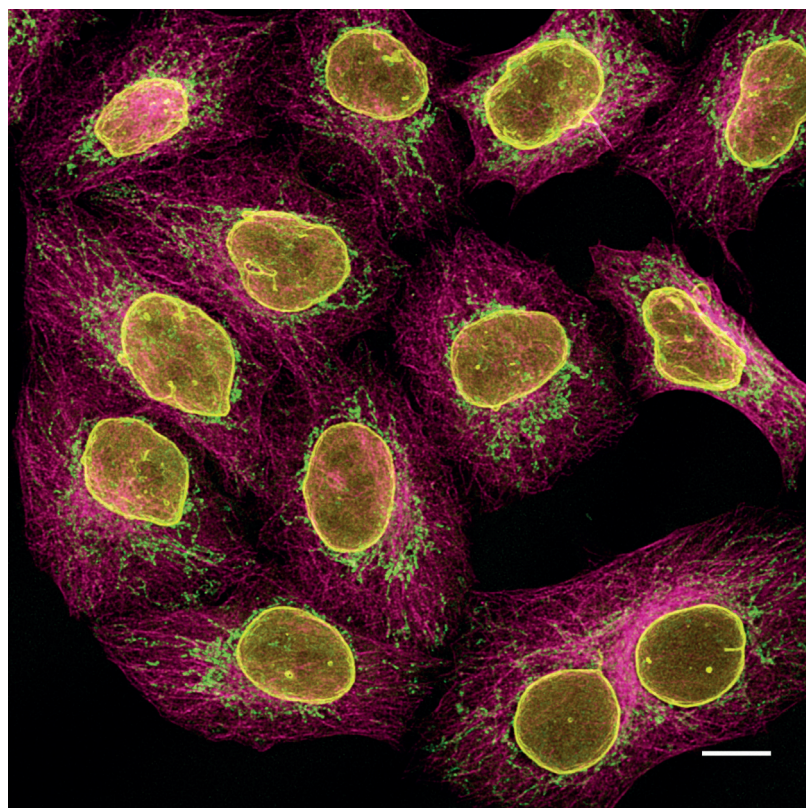
Cleaner Images

IgG applied to membrane

Subclass specific probe Alexa Fluor® 647 conjugated	Mouse IgG1	Mouse IgG2a	Mouse IgG2b	Mouse IgG2c	Mouse IgG3	Rabbit IgG
Alpaca α-mouse IgG1 V_HH	●					
Alpaca α-mouse IgG2b V_HH			●			
Alpaca α-mouse IgG3 V_HH					●	
Alpaca α-rabbit IgG V_HH						●
Competitor goat α-rabbit IgG antibody, pre-adsorbed	●	●	●	●	●	●

Nano-Secondaries are subclass-specific and do not cross-react with IgGs from other commonly used species. This high specificity in combination with low background enables multiplexing.
Note competitor's cross-reactivity to mouse IgGs despite of pre-adsorption against mouse serum.

Nano-Secondaries are recombinantly manufactured for reproducible results & unlimited supply

lubio science

Contact us for ordering or additional information
info@lubio.ch – www.lubio.ch
LubioScience GmbH – Baumackerstrasse 24 – 8050 Zürich – 041 417 02 80

Nano-Secondaries: Alpaca anti-rabbit recombinant V$_H$Hs

Multiplexing of HeLa cells with alpaca anti-rabbit and anti-mouse Nano-Secondaries.

Yellow: rabbit anti-Lamin + alpaca anti-rabbit IgG V$_H$H Alexa Fluor 568.
Green: mouse IgG1 anti-COX4 + alpaca anti-mouse IgG1 V$_H$H Alexa Fluor 488.
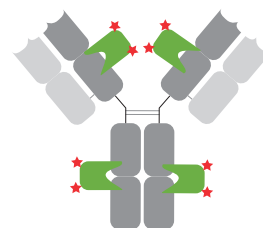*Magenta: mouse IgG2b anti-Tubulin + alpaca anti-mouse IgG2b V$_H$H Alexa Fluor 647. Scale bar, 10 µm.*

|	
Product name:	**Alpaca anti-rabbit IgG, recombinant V$_H$H**
Defined staining and conjugation: ▸ Site specific binding ▸ Stoichiometric labeling grey: primary antibody green: Nano-Secondaries red star: Alexa Fluor®	
Target/ Specificity:	Rabbit IgG
Host:	Alpaca, recombinantly produced
Format:	V$_H$H/ Nanobody
Clonality:	Mixture of 2 monoclonal V$_H$Hs, Fab- and Fc-specific
No cross-reactivity to:	Mouse, rat, sheep, goat, and guinea pig serum
Cross-reactivity to:	Human and macaque serum
Conjugates:	Alexa Fluor® 488, 568, 647
Applications:	▸ Immunofluorescence ▸ Super-resolution microscopy ▸ Western blotting

Immunofluorescence/ confocal microscopy of HeLa cells with alpaca anti-rabbit Nano-Secondaries. *Scale bar, 20 µm.*
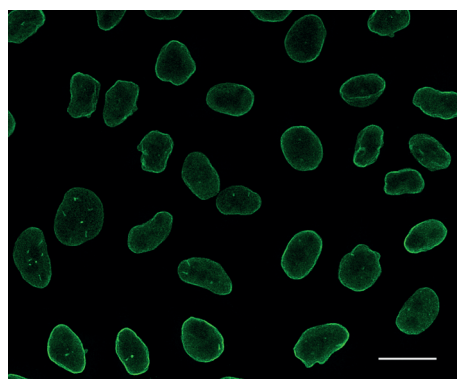
Rabbit anti-Lamin B1
+ alpaca anti-rabbit IgG
V$_H$H Alexa Fluor 488.
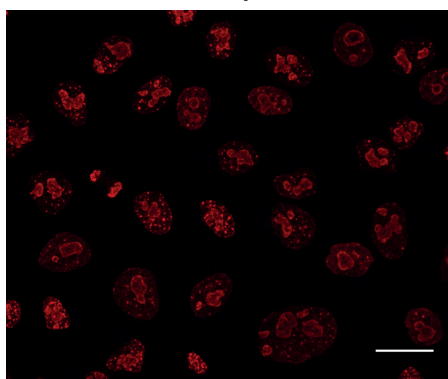
Rabbit anti-Ki67
+ alpaca anti-rabbit IgG
V$_H$H Alexa Fluor 568.
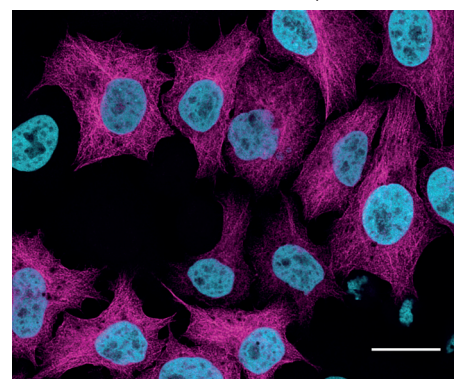
Rabbit anti-GFP (ChromoTek PABG1, Tubulin-GFP) + alpaca anti-rabbit IgG V$_H$H Alexa Fluor 647 (magenta)
+ H2B-RFP (cyan).

Order information

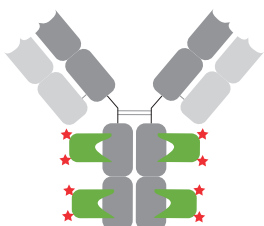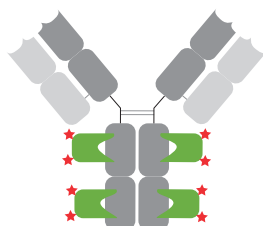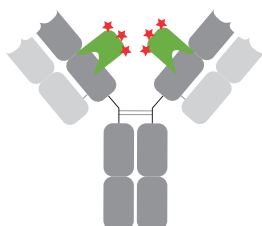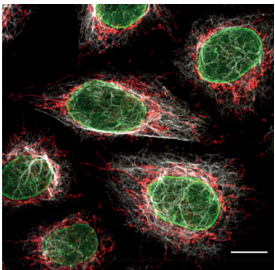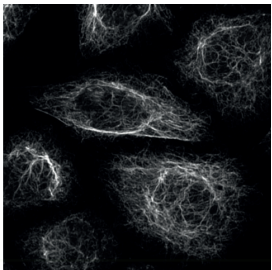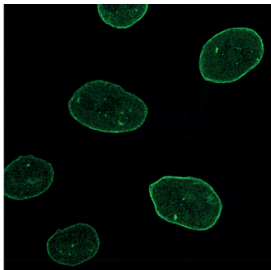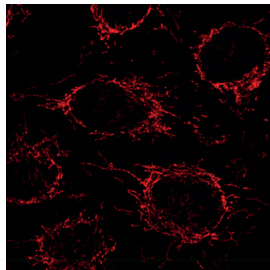| Product name	Alexa Fluor® 488	Alexa Fluor® 568	Alexa Fluor® 647
Alpaca anti-rabbit IgG, recombinant V$_H$H	10 µL: srbAF488-1-10	10 µL: srbAF568-1-10	10 µL: srbAF647-1-10
	100 µL: srbAF488-1-100	100 µL: srbAF568-1-100	100 µL: srbAF647-1-100

Order online:
https://shop.chromotek.com/

Order by email: order@chromotek.com
USA: usaorder@chromotek.com

Order by fax: +49 89 124 148 811
USA: 631 501 1060

info@chromotek.com   **www.chromotek.com**

Nano-Secondaries: Alpaca anti-mouse recombinant V$_H$Hs

| Product name:	Alpaca anti-mouse IgG1, Fc-specific recombinant V$_H$H	Alpaca anti-mouse IgG2b, Fc-specific recombinant V$_H$H	Alpaca anti-mouse IgG3, Fab-specific recombinant V$_H$H
Defined staining and conjugation: ▸ Site specific binding ▸ Stoichiometric labeling grey: primary antibody green: Nano-Secondaries red star: Alexa Fluor®			
Target/ Specificity:	Mouse IgG1	Mouse IgG2b	Mouse IgG3
Host:	Alpaca, recombinantly produced	Alpaca, recombinantly produced	Alpaca, recombinantly produced
Format:	V$_H$H/ Nanobody	V$_H$H/ Nanobody	V$_H$H/ Nanobody
Clonality:	Mixture of 2 monoclonal V$_H$Hs, Fc-specific	Mixture of 2 monoclonal V$_H$Hs, Fc-specific	Monoclonal V$_H$H, Fab-specific
No cross-reactivity to:	Rabbit, rat, sheep, goat, guinea pig, human, and macaque serum	Rabbit, rat, sheep, goat, guinea pig, human, and macaque serum	Rabbit, rat, sheep, goat, guinea pig, human, and macaque serum
Conjugates:	Alexa Fluor® 488, 568, 647	Alexa Fluor® 488, 568, 647	Alexa Fluor® 647
Applications:	▸ Immunofluorescence ▸ Super-resolution microscopy ▸ Western blotting	▸ Immunofluorescence ▸ Super-resolution microscopy ▸ Western blotting	▸ Immunofluorescence ▸ Super-resolution microscopy ▸ Western blotting

Multiplexing. Scale bar, 10 µm.

Mouse IgG1 anti-Vimentin + alpaca anti-mouse IgG1 V$_H$H Alexa Fluor 647.

Mouse IgG2b anti-Lamin + alpaca anti-mouse IgG2b V$_H$H Alexa Fluor 488.

Mouse IgG3 anti-MOT + alpaca anti-mouse IgG3 V$_H$H Alexa Fluor 568.
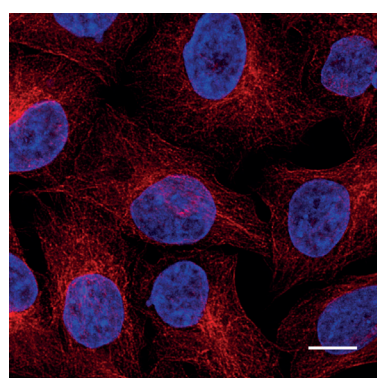
Immunostaining of HeLa cells with alpaca anti-mouse Nano-Secondary.
Mouse IgG2b anti-Tubulin + alpaca anti-mouse IgG2b V$_H$H Alexa Fluor 568. Nuclei were stained with DAPI (blue). Scale bar, 10 µm.
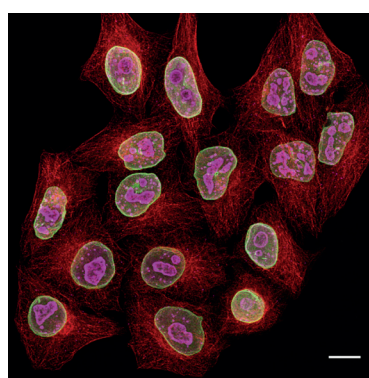
Multiplexing of HeLa cells with alpaca anti-mouse Nano-Secondaries and conventional secondary antibody.
Green: mouse IgG3 anti-Lamin + alpaca anti-mouse IgG3 V$_H$H Alexa Fluor 488, red: mouse IgG1 anti-Tubulin + alpaca anti-mouse IgG2b V$_H$H Alexa Fluor 568, magenta: rabbit anti-Ki67 + conventional polyclonal secondary anti-rabbit-AF647. Scale bar, 10 µm.
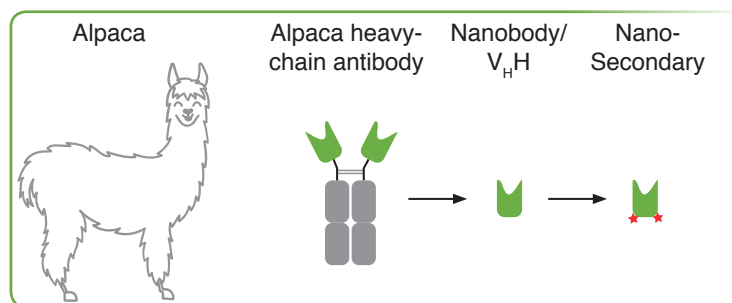
Order information

Product name	Alexa Fluor® 488	Alexa Fluor® 568	Alexa Fluor® 647
Alpaca anti-mouse IgG1, Fc-specific recombinant V$_H$H	10 µL: sms1AF488-1-10 100 µL: sms1AF488-1-100	10 µL: sms1AF568-1-10 100 µL: sms1AF568-1-100	10 µL: sms1AF647-1-10 100 µL: sms1AF647-1-100
Alpaca anti-mouse IgG2b, Fc-specific recombinant V$_H$H	10 µL: sms2bAF488-1-10 100 µL: sms2bAF488-1-100	10 µL: sms2bAF568-1-10 100 µL: sms2bAF568-1-100	10 µL: sms2bAF647-1-10 100 µL: sms2bAF647-1-100
Alpaca anti-mouse IgG3, Fab-specific recombinant V$_H$H	-	-	10 µL: sms3AF647-1-10 100 µL: sms3AF647-1-100

Order online:
https://shop.chromotek.com/

Order by email: order@chromotek.com
USA: usaorder@chromotek.com

Order by fax: +49 89 124 148 811
USA: 631 501 1060

info@chromotek.com   **www.chromotek.com**

Nano-Secondaries technology

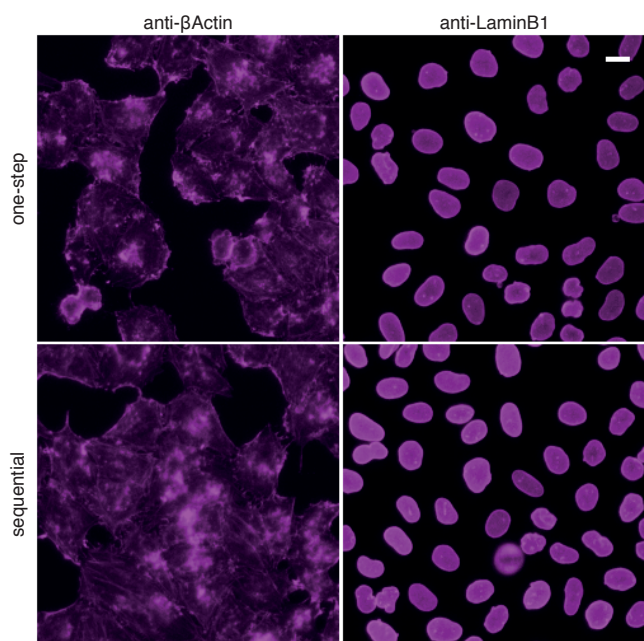| Alpaca | Alpaca heavy-chain antibody | Nanobody/ V_HH | Nano-Secondary |

Nanobodies/ V_HHs

Next to conventional IgG antibodies, alpacas also possess heavy chain only IgGs. These antibodies lack the C_H1 domain of the heavy chain and are devoid of any light chain. The antigen binding domain of the heavy chain only IgGs is called V_HH or Nanobody. Nano-Secondaries are IgG specific Nanobodies that are coupled to Alexa Fluor dyes.

High specificity and low background

During product development, we exclude Nanobodies that cross-react to other commonly used species' IgGs. We select only Nanobodies with the desired specificity. Therefore, our Nano-Secondaries have a very low background and do not require any kind of pre-adsorption.

One-step immunostaining

ChromoTek's Nano-Secondaries are monovalent and bind with very high affinity. Hence, they can be simultaneously incubated with the primary antibody. This method saves incubation time and washing steps.
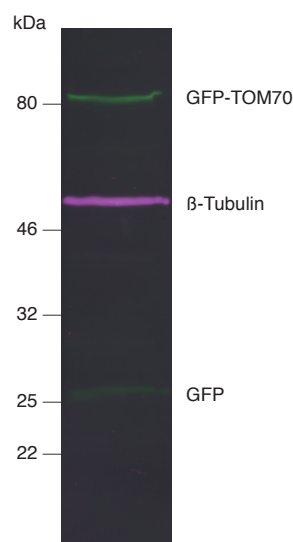
Multiplex fluorescent Western blotting

Nano-Secondaries can be applied in parallel in multiplex fluorescent Western blotting. This allows multiple targets to be analyzed simultaneously on the same blot at the same time. It is not necessary to strip and re-probe the Western blot membrane.

One-step immunostaining vs. sequential immunostaining of HeLa cells.
Primary antibodies + secondary alpaca anti-rabbit IgG V_HH Alexa Fluor® 647. Scale bar, 20 µm.

Multiplex fluorescent Western blot of GFP-TOM70, ß-Tubulin, and GFP in HEK293T cell lysate. Membrane was simultaneously incubated with primary antibodies and Nano-Secondaries.
Green: rabbit anti-GFP (ChromoTek PABG1) + alpaca anti-rabbit IgG V_HH Alexa Fluor 488.
Magenta: mouse anti-ß-Tubulin + alpaca anti-mouse IgG2b V_HH Alexa Fluor 647.

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.

ChromoTek is a registered trademark of ChromoTek GmbH. Nanobody is a registered trademark of Ablynx, a Sanofi company. Alexa Fluor® is a registered trademark of Life Technologies Corporation, a part of Thermo Fisher Scientific Inc.

Lubioscience

Contact us for ordering or additional information
info@lubio.ch – www.lubio.ch
LubioScience GmbH – Baumackerstrasse 24 – 8050 Zürich – 041 417 02 80

info@chromotek.com **www.chromotek.com**