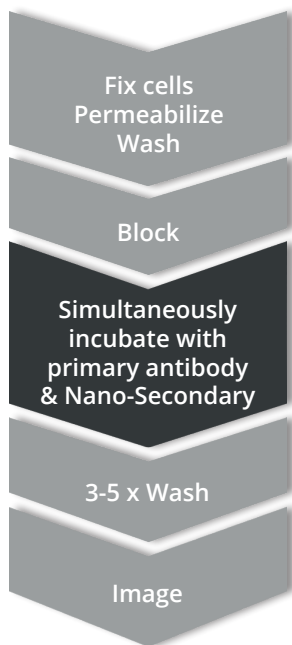


## Nano-Secondaries: The next level of secondary antibodies

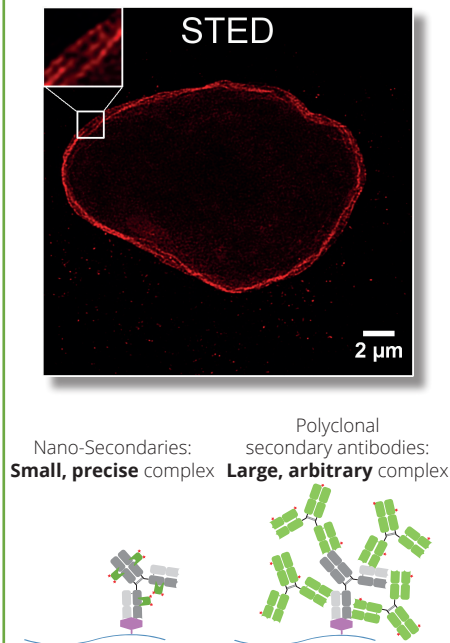
Nano-Secondaries are secondary antibodies. They are based on monoclonal recombinant V<sub>H</sub>H/ Nanobodies. Nano-Secondaries bind to primary antibodies with high affinity and specificity. Our Nano-Secondaries are conjugated to Alexa Fluor® dyes. Currently, Nano-Secondaries against rabbit and mouse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

### Faster Staining



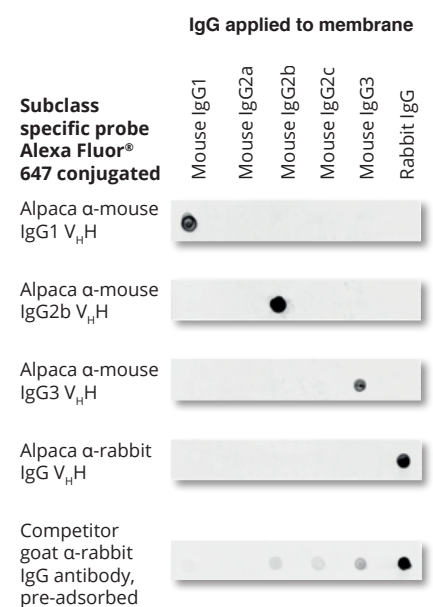
One-step immunostaining is the simultaneous incubation of primary antibody and Nano-Secondary. This method reduces incubation and hands-on time. Simultaneous incubation also supports multiplexing, tissue penetration, and cell staining for flow cytometry.

### Higher Resolution



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

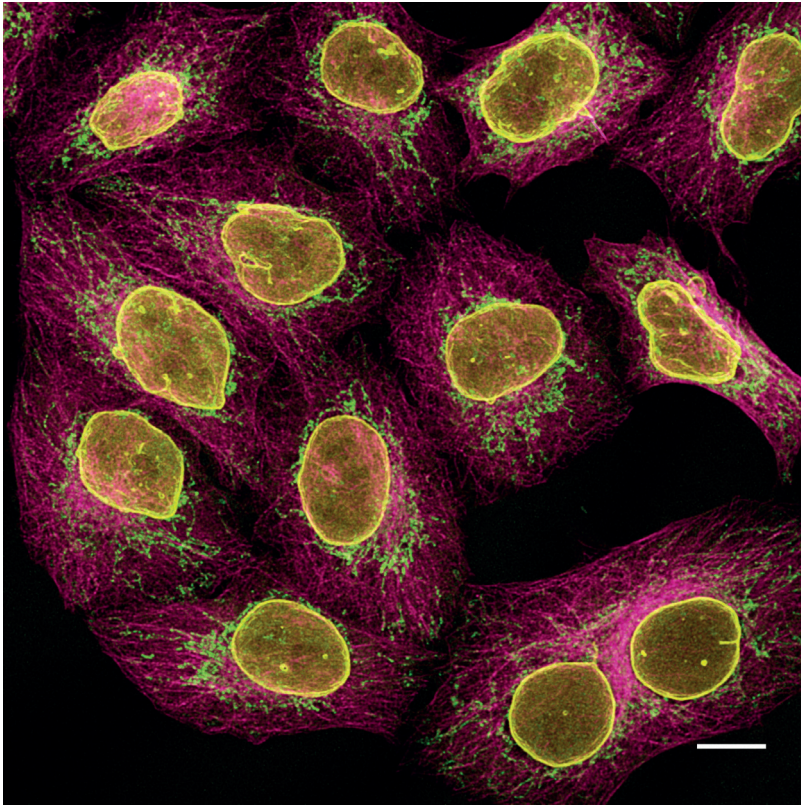


Nano-Secondaries are subclass-specific and do not cross-react with IgGs from other commonly used species. This high specificity in combination with low background enables multiplexing.

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

# Nano-Secondaries: Alpaca anti-rabbit recombinant V<sub>H</sub>Hs



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

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

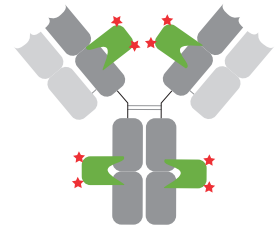
Product name:

**Alpaca anti-rabbit IgG,  
recombinant V<sub>H</sub>H**

Defined staining and conjugation:

- ▶ Site specific binding
- ▶ Stoichiometric labeling

grey: primary antibody  
 green: Nano-Secondaries  
 red star: Alexa Fluor®

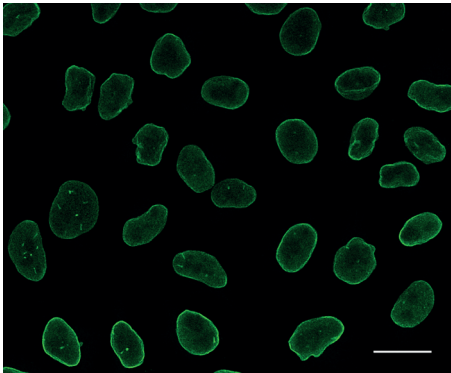


Target/ Specificity:	Rabbit IgG
Host:	Alpaca, recombinantly produced
Format:	V <sub>H</sub> H/ Nanobody
Clonality:	Mixture of 2 monoclonal V <sub>H</sub> Hs, Fab- and Fc-specific
No cross-reactivity to:	Mouse, rat, sheep, goat, and guinea pig serum
Cross-reactivity to:	Human and macaque serum
Conjugates:	Alexa Fluor® 488, 568, 647

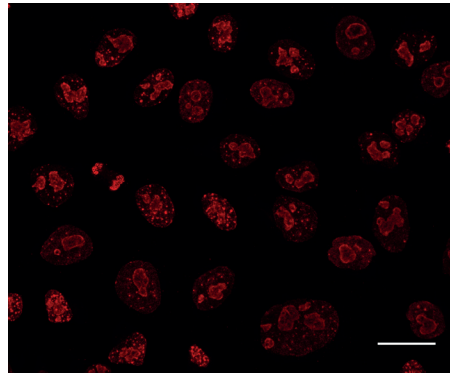
Applications:

- ▶ Immunofluorescence
- ▶ Super-resolution microscopy
- ▶ Western blotting

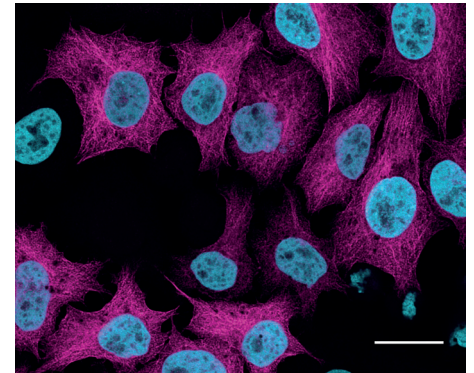
## Immunofluorescence/ confocal microscopy of HeLa cells with alpaca anti-rabbit Nano-Secondaries. Scale bar, 20 μm.



Rabbit anti-Lamin B1 + alpaca anti-rabbit IgG V<sub>H</sub>H Alexa Fluor 488.



Rabbit anti-Ki67 + alpaca anti-rabbit IgG V<sub>H</sub>H Alexa Fluor 568.



Rabbit anti-GFP (ChromoTek PABG1, Tubulin-GFP) + alpaca anti-rabbit IgG V<sub>H</sub>H Alexa Fluor 647 (magenta) + H2B-RFP and RFP-Booster (ChromoTek rba549, cyan).

## Order information

Product name	Alexa Fluor® 488	Alexa Fluor® 568	Alexa Fluor® 647
Alpaca anti-rabbit IgG, recombinant V <sub>H</sub> H	10 μL: srbAF488-1-10 100 μL: srbAF488-1-100	10 μL: srbAF568-1-10 100 μL: srbAF568-1-100	10 μL: srbAF647-1-10 100 μL: srbAF647-1-100

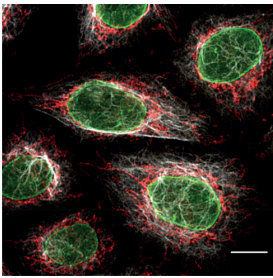
Order online:  
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Order by email: [order@chromotek.com](mailto:order@chromotek.com)  
 USA: [usaorders@chromotek.com](mailto:usaorders@chromotek.com)

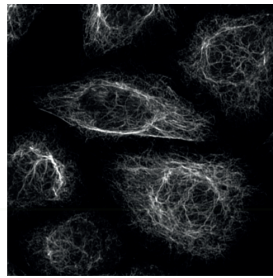
Order by fax: +49 89 124 148 811  
 USA: 631 501 1060

# Nano-Secondaries: Alpaca anti-mouse recombinant V<sub>H</sub>Hs

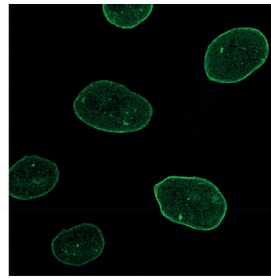
Product name:	Alpaca anti-mouse IgG1, Fc-specific recombinant V <sub>H</sub> H	Alpaca anti-mouse IgG2b, Fc-specific recombinant V <sub>H</sub> H	Alpaca anti-mouse IgG3, Fab-specific recombinant V <sub>H</sub> H
Defined staining and conjugation: <ul style="list-style-type: none"> <li>▶ Site specific binding</li> <li>▶ Stoichiometric labeling</li> </ul> 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 <sub>H</sub> H/ Nanobody	V <sub>H</sub> H/ Nanobody	V <sub>H</sub> H/ Nanobody
Clonality:	Mixture of 2 monoclonal V <sub>H</sub> Hs, Fc-specific	Mixture of 2 monoclonal V <sub>H</sub> Hs, Fc-specific	Monoclonal V <sub>H</sub> 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:	<ul style="list-style-type: none"> <li>▶ Immunofluorescence</li> <li>▶ Super-resolution microscopy</li> <li>▶ Western blotting</li> </ul>	<ul style="list-style-type: none"> <li>▶ Immunofluorescence</li> <li>▶ Super-resolution microscopy</li> <li>▶ Western blotting</li> </ul>	<ul style="list-style-type: none"> <li>▶ Immunofluorescence</li> <li>▶ Super-resolution microscopy</li> <li>▶ Western blotting</li> </ul>



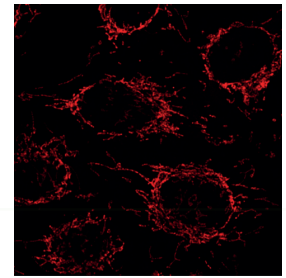
Multiplexing.  
Scale bar, 10 μm.



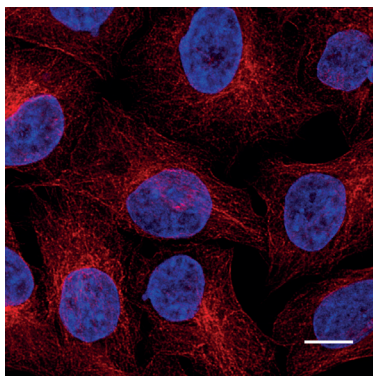
Mouse IgG1 anti-Vimentin + alpaca anti-mouse IgG1 V<sub>H</sub>H Alexa Fluor 647.



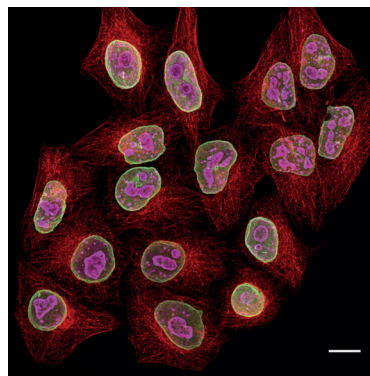
Mouse IgG2b anti-Lamin + alpaca anti-mouse IgG2b V<sub>H</sub>H Alexa Fluor 488.



Mouse IgG3 anti-MOT + alpaca anti-mouse IgG3 V<sub>H</sub>H Alexa Fluor 568.



**Immunostaining of HeLa cells with alpaca anti-mouse Nano-Secondary.**  
 Mouse IgG2b anti-Tubulin + alpaca anti-mouse IgG2b V<sub>H</sub>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<sub>H</sub>H Alexa Fluor 488, red: mouse IgG1 anti-Tubulin + alpaca anti-mouse IgG1 V<sub>H</sub>H Alexa Fluor 568, magenta: rabbit anti-Ki67 + conventional polyclonal secondary anti-rabbit-AF647. Scale bar, 10 μm.

**Order information**

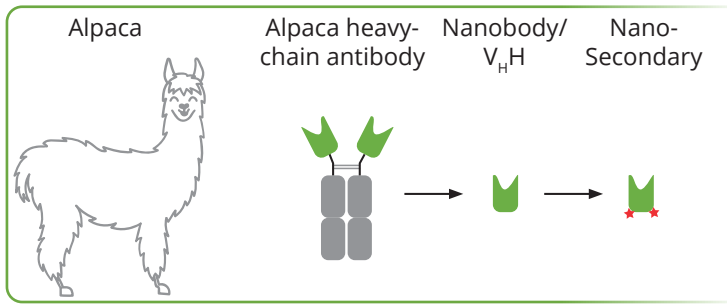
Product name	Alexa Fluor® 488	Alexa Fluor® 568	Alexa Fluor® 647
Alpaca anti-mouse IgG1, Fc-specific recombinant V <sub>H</sub> H	10 μL: sms1AF488-1-10 100 μL: sms1AF488-1-100	10 μL: sms1AF568-1-10 100 μL: sms1AF568-1-100	10 μL: sms1AF647-1-10 100 μL: sms1AF647-1-100
Alpaca anti-mouse IgG2b, Fc-specific recombinant V <sub>H</sub> 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 <sub>H</sub> H	-	-	10 μL: sms3AF647-1-10 100 μL: sms3AF647-1-100

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 USA: [usaorders@chromotek.com](mailto:usaorders@chromotek.com)

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

# Nano-Secondaries technology



## Nanobodies/ V<sub>H</sub>Hs

Next to conventional IgG antibodies, alpacas also possess heavy chain only IgGs. These antibodies lack the C<sub>H</sub>1 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<sub>H</sub>H or Nanobody. Nano-Secondaries are IgG specific Nanobodies that are coupled to Alexa Fluor dyes.

## High specificity and low background

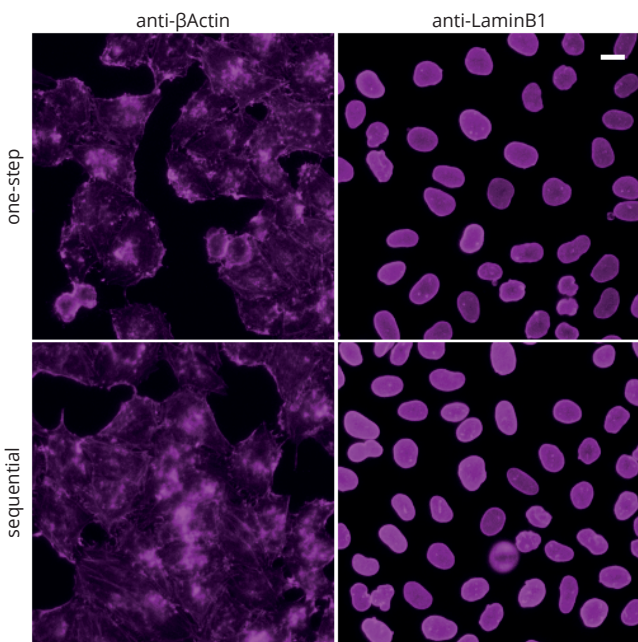
During product development, we exclude Nanobodies that cross-react to other commonly used species' IgGs. We select only Nanobodies with the desired specificity. Therefore, our Nano-Secondaries have a very low background and do not require any kind of pre-adsorption.

## 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.

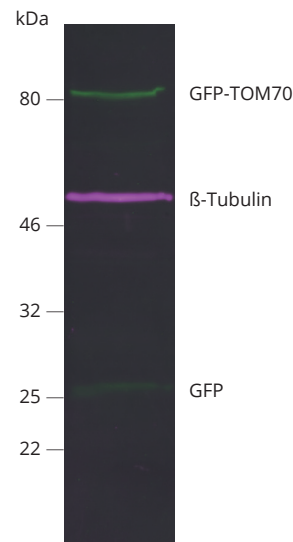
## Multiplex fluorescent Western blotting

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<sub>H</sub>H Alexa Fluor® 647. Scale bar, 20 μm.



**Multiplex fluorescent Western blot of GFP-TOM70, β-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<sub>H</sub>H Alexa Fluor 488. Magenta: mouse anti-β-Tubulin + alpaca anti-mouse IgG2b V<sub>H</sub>H 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.

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