

Application Note

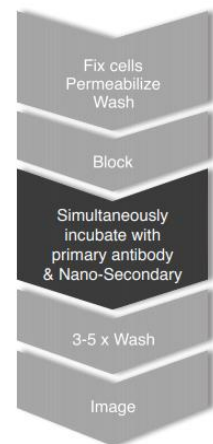
One-step immunostaining with Nano-Secondaries

Introduction: What is a Nano-Secondary?

ChromoTek's Nano-Secondaries are a novel class of secondary antibodies for higher resolution, cleaner images, and faster immunostaining. Nano-Secondaries consist of Nanobodies/ V_HHs that bind to primary antibodies with high affinity in a species and subclass specific manner. Nano-Secondaries are conjugated to Alexa Fluor® dyes.

Introduction: One-step immunostaining

Because the Nano-Secondaries are monovalent and bind with high specificity and affinity to their target IgGs, they can be simultaneously incubated with the primary antibody. This results in a one-step immunostaining. It saves incubation time and reduces washing steps, and hand-on time. Simultaneous incubation also supports multiplexing, live-cell immunostaining, and improves cell viability for flow cytometric analysis.



One-step staining protocol for immunofluorescence detection

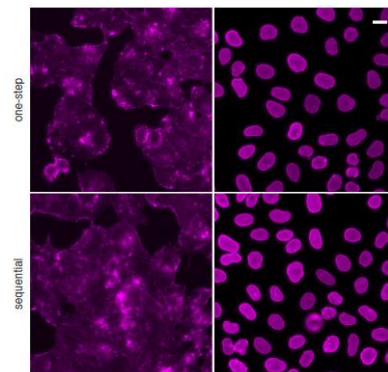
The protocol below provides guidelines for one-step immunostaining of cultured adherent mammalian cells. For immunostaining of other cell types, tissues or whole organs please adjust accordingly.

Note, the efficiency of one-step immunostaining depends on the primary antibody. Carefully follow the supplier's recommendations for the specific primary antibody you are using, especially regarding cell fixation (PFA or acetone/methanol), permeabilization reagent, blocking buffer composition, incubation time (1 h or overnight) and temperature (room temperature or 4°C). It helps a lot to pre-test your primary antibody with respect to their optimal dilution, isotype, and correct staining pattern using conventional two-step immunostaining before moving to a faster one-step protocol.

Step	Basic condition	Alternative condition
1. Fix cells	4% PFA-PBS – 10 min RT	(acetone/) methanol - 10 min on ice
2. Wash cells 3-5 times with PBS		

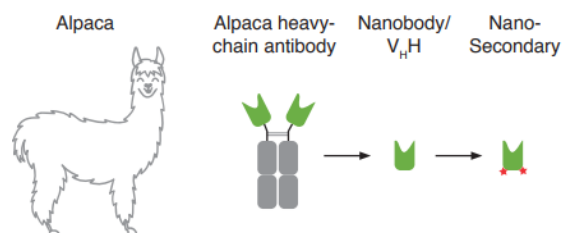
3. Permeabilize cells	0.1% - 0.5% Triton-X100 in PBS - 5 min RT	saponin, digitonin, NP40 in PBS or no permeabilization
4. Wash cells 3-5 times with PBS		
5. Block cells	4% BSA-PBS – 20 min RT	5% normal goat serum, dry milk, cold fish skin gelatin
6. Incubate cells with primary antibody and Nano-Secondary in blocking solution	1 h RT	overnight at 4°C; 15-30 min at 4°C if live-cell
7. Wash cells 3-5 times with PBS		
8. Counterstain with DAPI, mount, image		

Simultaneous one-step immunostaining vs. sequential immunostaining. HeLa cells were immunostained with different primary rabbit antibodies (left anti-Actin, right anti-Lamin) and secondary alpaca anti-rabbit IgG, recombinant V_HH, Alexa Fluor® 647 (1:1,000). Scale bar, 20 µm.



What is a Nanobody or V_HH?

In addition 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 light chains. Their antigen binding domain is built up solely by their heavy chain and is called V_HH or Nanobody. Nano-Secondaries are Nanobodies against rabbit IgG or mouse IgG subclasses that are chemically conjugated to Alexa Fluor dyes.



Products

Nano-Secondaries	Product Size	Product Code
Alpaca anti-rabbit IgG, recombinant VHH, Alexa Fluor® 488, 568, 647	10 µl	srb1AF488/568/647-1-10
	100 µl	srb1AF488/568/647-1-100
Alpaca anti-mouse IgG1, recombinant VHH, Alexa Fluor® 488, 568, 647	10 µl	sms1AF488/568/647-1-10
	100 µl	sms1AF488/568/647-1-100
Alpaca anti-mouse IgG2b, recombinant VHH, Alexa Fluor® 488, 568, 647	10 µl	sms2bAF488/568/647-1-10
	100 µl	sms2bAF488/568/647-1-100
Alpaca anti-mouse IgG3, recombinant VHH, Alexa Fluor® 647	10 µl	sms3AF647-1-10
	100 µl	sms3AF647-1-100

Related Products	Product Size	Product Code
Mouse monoclonal IgG1 antibody [28A] to Halo-tag	20 µl	28a8-20
	100 µl	28a8-100
GFP antibody rabbit polyclonal [PABG1]	20 µl	PABG1-20
	100 µl	PABG1-100

For product details, information, and ordering visit www.chromotek.com.

Contact: support@chromotek.com

lubio
science

Your distributor in Switzerland

LubioScience GmbH
Baumackerstrasse 24
8050 Zürich
Phone 041 417 02 80
Fax 041 417 02 89

info@lubio.ch
www.lubio.ch

For research use only. Not for diagnostic or therapeutic use.

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. Other suppliers' products may be trademarks or registered trademarks of the corresponding supplier each. Statements on other suppliers' products are given according to our best knowledge.