Application Note

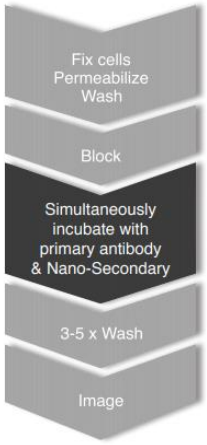
One-step immunostaining with Nano-Secondaries

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

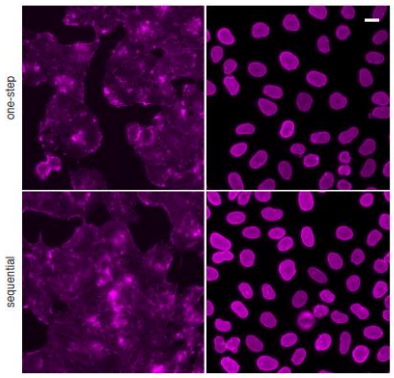
The protocol below provides guidelines for one-step immunostaining of cultured adherent mammalian cells. For immunostaining of other cell types, tissues or whole organs please adjust accordingly.

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.

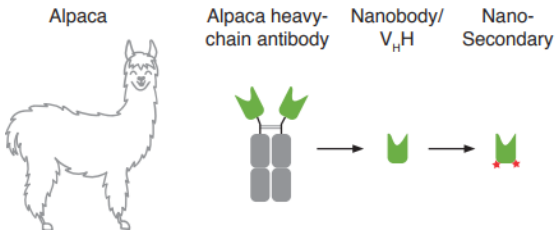
Step	Basic condition	Alternative condition
1. Fix cells	4% PFA-PBS – 10 min RT	(acetone/) methanol - 10 min on ice
2. Wash cells 3-5 times with PBS		

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 VHH, 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 100 µl	srb1AF488/568/647-1-10 srb1AF488/568/647-1-100
Alpaca anti-mouse IgG1, recombinant VHH, Alexa Fluor® 488, 568, 647	10 µl 100 µl	sms1AF488/568/647-1-10 sms1AF488/568/647-1-100
Alpaca anti-mouse IgG2b, recombinant VHH, Alexa Fluor® 488, 568, 647	10 µl 100 µl	sms2bAF488/568/647-1-10 sms2bAF488/568/647-1-100
Alpaca anti-mouse IgG3, recombinant VHH, Alexa Fluor® 647	10 µl 100 µl	sms3AF647-1-10 sms3AF647-1-100

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

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

Contact: support@chromotek.com

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