

Application note

One-step incubation with Nano-Secondaries[®]

Content

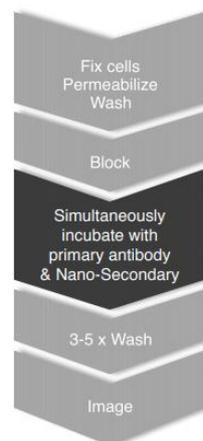
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1. What is a Nano-Secondary[®]?

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

2. One-step incubation

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 incubation. It saves incubation time and reduces washing steps, and hands-on time. Simultaneous incubation also supports multiplexing, live-cell immunostaining, and improves cell viability for flow cytometric analysis.



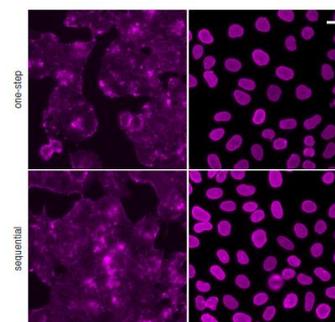
3. One-step incubation protocol for immunofluorescence detection

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

Note, the efficiency of one-step incubation 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 is recommended to pre-test your primary antibody with respect to its optimal dilution, isotype, and correct staining pattern using conventional two-step incubation before moving to a faster one-step protocol.

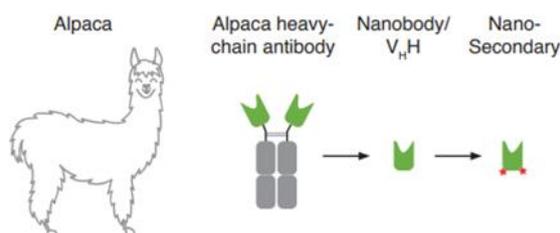
Step	Basic condition	Alternative condition
1. Fix cells	4% PFA-PBS – 10 min RT	Methanol or acetone – 10 min on ice
2. Wash cells	3-5 times with PBS	
3. Permeabilize cells	0.1-0.5% Triton-X100 in PBS – 5 min RT	Saponin, digitonin, NP-40 in PBS or no permeabilization
4. Wash cells	3-5 times with PBS	
5. Block cells	4% BSA in 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 +4°C; 15-30 min +4°C if live-cell
7. Wash cells	3-5 times with PBS	
8. Counterstain with DAPI, mount, image		

Simultaneous one-step incubation vs. sequential incubation. HeLa cells were immunostained with different primary rabbit antibodies (left anti-Actin, right anti-Lamin) and Nano-Secondary[®] alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor[®] 647 [CTK0101, CTK0102] (1:1,000). Scale bar, 20 µm.



4. What is a Nanobody or V_HH?

In addition to conventional IgG antibodies, alpacas also possess heavy chain only IgGs. These antibodies lack the CH1 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 human IgG, rabbit IgG or mouse IgG subclasses that are chemically conjugated to Alexa Fluor dyes.

5. Product overview

Nano-Secondaries (Alexa Fluor conjugated)	Product size	Product code
Nano-Secondary [®] alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor [®] 488/568/647 [CTK0101, CTK0102]	10 µL 100 µL	srbAF488/568/647-1-10 srbAF488/568/647-1-100
Nano-Secondary [®] alpaca anti-mouse IgG1, recombinant VHH, Alexa Fluor [®] 488/568/647 [CTK0103, CTK0104]	10 µL 100 µL	sms1AF488/568/647-1-10 sms1AF488/568/647-1-100
Nano-Secondary [®] alpaca anti-mouse IgG2b, recombinant VHH, Alexa Fluor [®] 488/568/647 [CTK0105, CTK0106]	10 µL 100 µL	sms2bAF488/568/647-1-10 sms2bAF488/568/647-1-100
Nano-Secondary [®] alpaca anti-mouse IgG3, recombinant VHH, Alexa Fluor [®] 647 [CTK0107]	10 µL 100 µL	sms3AF647-1-10 sms3AF647-1-100
Nano-Secondaries (unconjugated)	Product size	Product code
Nano-Secondary [®] alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, for 2x Cys conjugation [CTK0101]	250 µL	srbGCys2-1-500
Nano-Secondary [®] alpaca anti-rabbit IgG, recombinant VHH, for 2x Cys conjugation [CTK0102]	250 µL	srbGCys2-2-500
Nano-Secondary [®] alpaca anti-mouse IgG1, recombinant VHH, for 2x Cys conjugation [CTK0103]	250 µL	smsG1Cys2-1-500
Nano-Secondary [®] alpaca anti-mouse IgG1, recombinant VHH, for 2x Cys conjugation [CTK0104]	250 µL	smsG1Cys2-2-500
Nano-Secondary [®] alpaca anti-mouse IgG2b, recombinant VHH, for 2x Cys conjugation [CTK0105]	250 µL	smsG2bCys2-1-500
Nano-Secondary [®] alpaca anti-mouse IgG2b, recombinant VHH, for 2x Cys conjugation [CTK0106]	250 µL	smsG2bCys2-2-500
Related Products	Product size	Product code
GFP antibody rabbit polyclonal [PABG1]	20 µL 100 µL	PABG1-20 PABG1-100
Halo antibody [28A8]	20 µL 100 µL	28a8-20 28a8-100
V5-tag antibody [SV5-P-K]	20 µL 200 µL	v5ab-20 v5ab-200

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

6. Disclaimer

Only for research applications, not for diagnostic or therapeutic use!

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