## For Research Use Only

## Nano-Secondary® anti-mouse IgG1, recombinant VHH, Alexa Fluor® 647 [CTK0103, CTK0104]



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Catalog Number: sms1AF647-1 3 Publications

Catalog Number: sms1AF647-1 **Basic Information** 

**Applications:** IF, WB, FC Host: Alpaca Conjugate: Alexa Fluor® 647

RRID: AB\_2827579

**Purification Method:** Recombinant expression, affinity purification

Class:

Recombinant

Type:
Mixture of 2 monoclonal Nanobodies

 $Nano-Secondary @ \ anti-mouse \ lgG1, Fc-specific \ recombinant \ VHH \ reagent \ uses \ a \ novel \ class \ of \ anti-mouse \ lgG1 \ sotype \ specific \ antibodies. This secondary \ antibody \ product \ consists \ of \ Nanobodies \ that \ bind \ to \ mouse \ lgG1 \ with \ high \ affinity \ \mathcal{B}_{normal \ normal \$ Description

specificity.

**Species Reactivity** Mouse IgG1 Fc-fragment

No cross-reactivity: goat, guinea pig, rabbit, rat, sheep, human, macaque serum proteins, mouse IgG2a, IgG2b, IgG2c, IgG3

Physical State Liquid

**Suggested Dilution** Immunofluorescence 1:500 Super-resolution microscopy 1:500

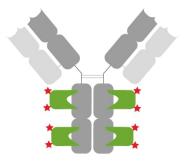
Western blot 1:1,000

Affinity (K<sub>D</sub>) CTK0103:  $K_D = 0.13$  nM, CTK0104:  $K_D = 0.63$ nM

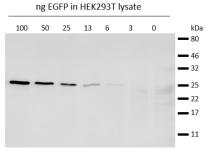
Storage Store at -20°C short term or -80°C long term. Aliquot upon delivery. Avoid freeze-thaw cycles.

10 mM HEPES pH 7.0, 500 mM NaCl, 5 mM EDTA, Preservative: 0.09 % Sodium azide

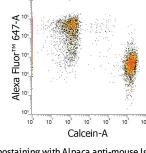
## Selected Validation Data



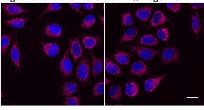
Anti-mouse IgG1 Nano-Secondary: Well-defined and characterized immunostaining. Primary antimouse IgG1 antibody (grey) with 2½2 monoclonal mouse Fc- specific Nanobodies (green) bound. In total, 8 fluorophores (red stars) label the mouse IgG1 primary antibody.



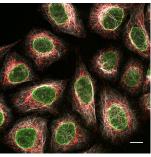
Western blot analysis of EGFP (EGFP-250, ChromoTek) added to HEK293T cell lysate. Detection with anti-GFP mouse IgG 1 antibody and alpaca anti-mouse IgG1 VHH Alexa Fluor® 647.



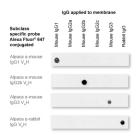
Immunostaining with Alpaca anti-mouse IgG1 Alexa Fluor 647 Nano-Secondaries for FACS. Jurkat CD3+ and Jurkat CD3- cell lines were mixed and immunostained live with anti-CD3 mouse IgG1+ alpaca anti-mouse IgG1 VHH Alexa Fluor 647 (1:600). CD3- cells were pre-stained with Calcein. Two cell populations can be clearly distinguished on the dot-plot: Alexa Fluor 647-positive Calceinnegative cells and Alexa Fluor 647-negative



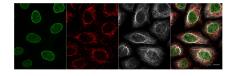
One-step staining (left) vs. sequential staining (right) of HeLa cells with anti-COX4 (mitochondria) mouse IgG1 monoclonal primary antibody + alpaca anti-mouse IgG1 VHH Alexa Fluor® 647 (magenta). Cell nuclei are stained with DAPI (blue). Scale bar, 20 µm.



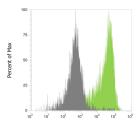
Multiplexed immunostaining of HeLa cells with 3 subclass-specific alpaca anti-mouse Nano-Secondaries. Grey: Mouse IgG1 anti-Vimentin + alpaca anti-mouse IgG1VHH Alexa Fluor® 647. Green: Mouse IgG2b anti-Lamin + alpaca anti-mouse IgG2b VHH Alexa Fluor® 488. Red: Mouse IgG3 anti-MOT + alpaca anti-mouse IgG3 VHH Alexa Fluor® 568. Scale bar, 10 µm. Images were recorded at the Core Facility Bioimaging at the Biomedic



The anti-mouse IgG1 Nano-Secondary is subclassspecific and does not cross-react with IgGs from other commonly used species (here rabbit) and with mouse IgG2b and IgG3 subclasses.

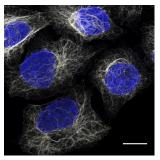


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Alexa Fluor™ 647 - RL1-A

Flow cytometry with anti-mouse Nano-Secondaries. Jurkat CD3+ and Jurkat CD3- cell lines were mixed and immunostained live with anti-CD3 mouse IgG1 and alpaca anti-mouse IgG1 VHH Alexa Fluor® 647 (1:600). Two cell populations can be clearly distinguished with a 2-log shift: CD3-positive cells



HeLa cells were immunostained with anti-Vimentin mouse IgG1 antibody and alpaca anti-mouse IgG1 VHH Alexa Fluor® 647 (grey). Nuclei were stained with DAPI, blue. Scale bar, 10 µm. Images were recorded at the Core Facility Bioimaging at the Biomedical Center, LMU Munich.



One-step immunostaining is the simultaneous incubation of mouse IgG1 primary antibody and anti-mouse IgG1 Nano-Secondary. This method reduces incubation and hands-on time. Simultaneous incubation also supports multiplexing, tissue penetration, and cell staining for flow cytometry.