# Nano-Secondary® alpaca anti-mouse IgG1, recombinant VHH, for 2x Cys conjugation [CTK0103]

Product code: smsG1Cys2-1

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## Properties

| | |
|---|---|
| Description | Monovalent, recombinant secondary single domain antibody to mouse IgG1: alpaca monoclonal Nanobody, Fc-specific, for 2x Cys conjugation |
| Product Type | Nano-Secondary®, secondary Nanobody (VHH) |
| Format | Alpaca single domain antibody, monovalent |
| Host | Alpaca-derived, recombinantly produced in bacteria |
| Target / Specificity | Fc-fragment of mouse IgG1 |
| Cross-reactivity | No cross-reactivity to goat, guinea pig, human, macaque, rabbit, rat, and sheep serum and to mouse IgG2a, IgG2b, IgG2c, and IgG3 |
| Immunogen | Purified mouse IgG1 |
| Clonality | Monoclonal Nanobody |
| Conjugate chemistry | N- and C-terminal cysteine conjugation with thiol-reactive reagents, e.g. maleimides |
| Clone | CTK0103 |
| Molecular weight | 14.3 kDa |
| Extinction coefficient (280 nm) | 25,690 $M^{-1} {*} cm^{-1}$ |
| Affinity (*Kd*) of unconjugated Nano-Secondary | *Kd* = 0.1 nM |
| Concentration | 2 mg/mL |
| Purity | Recombinantly expressed and purified |
| Form | Buffered aqueous solution |
| Validation | Application validated for maleimide conjugation. Fluorophore conjugates of Nano-Secondaries can be used in immunofluorescence, flow cytometry and Western blotting. Determination of cross-reactivity, sequence, affinity, and melting point. |
| Synonyms | Alpaca single domain antibody, VHH, Nanobody, binding domain of single domain antibody, Nano-antibody |
| Storage buffer | 10 mM HEPES pH 7.0, 500 mM NaCl, 1 mM TCEP Preservative: 0.09 % sodium azide, safety datasheet (SDS): sodium azide |

| Storage instructions | Shipped on dry ice. Store at -20°C/-4°F. Avoid freeze-thaw cycles. |
|---|---|
| Size | 500 µg |
| RRID | AB_2864263 |

# Cysteine labeling protocol

This protocol provides recommendations for the site-directed labeling of ChromoTek Nanobodies containing 2 ectopic cysteines with thiol-reactive fluorescent dyes by maleimide chemistry.

## General considerations and recommendations

- Each fluorescent dye is different and can influence the Nanobody to a different extent. The conditions for labelling must be established individually for each dye.
- Remember that Nanobodies are only 1/10 of the size of an antibody when antibody labeling kits are used.
- Many fluorescent dyes have a hydrophobic structure. The conjugation of hydrophobic dyes to Nanobodies can affect the solubility of the Nanobody.

## Preparation of dye

- Follow the dye manufacturer's protocol.
- Freshly prepare the dye stock solution immediately before starting the labeling reaction. Functional groups lose their reactivity during storage.
- Adjust the molar excess of the dye according to the dye manufacturer's recommendations. Use at least 2 equivalents of dye per Nanobody (corresponds to 1 equivalent of dye per cysteine) to ensure complete labeling of both cysteines. A greater excess of the dye may be needed depending on the reactivity of the dye.
- Dyes are dissolved in organic solvents. Note that organic solvents can affect the stability and can facilitate precipitation of the Nanobody.

## Preparation of Nanobody

- Centrifuge material before use (20,000x g, 15 min, +4°C).
- Nanobodies are stored in HEPES buffer (10 mM HEPES pH 7.0, 500 mM NaCl, 1 mM TCEP) which is compatible with many dyes and labeling protocols. An additional buffer exchange step is not necessary.
- Note that the labeling buffer can influence the labeling efficiency.

## Conjugation reaction

- Mix the diluted dye with the Nanobody.
- Place the tube on ice and incubate for 1-2 h.
- Optional: Overlay the labeling reaction with argon.

## Removal of unbound dye

- Centrifuge the solution after the labeling reaction is completed (20,000x g, 15 min, +4°C) and continue working with the supernatant.
- Separate unbound dye from the labeled Nanobody by one of the following options or by a combination thereof:
  - Size exclusion column (length: >30 cm)
  - Dialysis (molecular weight cut off: 3.5 kDa)
  - Spin column (molecular weight cut off: 7 kDa)
  - Desalting column

## Storage

- Aliquot the labeled Nanobody and store at -20°C. Avoid freeze-thaw cycles. Protect from light.
- Add 0.1% sodium azide for long-term storage to prevent bacterial contamination.

# Product overview and related products

| Nano-Secondaries (Alexa Fluor conjugated) | Product code |
|---|---|
| Nano-Secondary® alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor® 488 [CTK0101, CTK0102] | srbAF488-1-10; -100 |
| Nano-Secondary® alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor® 568 [CTK0101, CTK0102] | srbAF568-1-10; -100 |
| Nano-Secondary® alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, Alexa Fluor® 647 [CTK0101, CTK0102] | srbAF647-1-10; -100 |
| Nano-Secondary® alpaca anti-mouse IgG1, recombinant VHH, Alexa Fluor® 488 [CTK0103, CTK0104] | sms1AF488-1-10; -100 |
| Nano-Secondary® alpaca anti-mouse IgG1, recombinant VHH, Alexa Fluor® 568 [CTK0103, CTK0104] | sms1AF568-1-10; -100 |
| Nano-Secondary® alpaca anti-mouse IgG1, recombinant VHH, Alexa Fluor® 647 [CTK0103, CTK0104] | sms1AF647-1-10; -100 |
| Nano-Secondary® alpaca anti-mouse IgG2b, recombinant VHH, Alexa Fluor® 488 [CTK0105, CTK0106] | sms2bAF488-1-10; -100 |
| Nano-Secondary® alpaca anti-mouse IgG2b, recombinant VHH, Alexa Fluor® 568 [CTK0105, CTK0106] | sms2bAF568-1-10; -100 |
| Nano-Secondary® alpaca anti-mouse IgG2b, recombinant VHH, Alexa Fluor® 647 [CTK0105, CTK0106] | sms2bAF647-1-10; -100 |
| Nano-Secondary® alpaca anti-mouse IgG3, recombinant VHH, Alexa Fluor® 647 [CTK0107] | sms3AF647-1-10; -100 |

| Nano-Secondaries (unconjugated) | |
|---|---|
| Nano-Secondary® alpaca anti-human IgG/anti-rabbit IgG, recombinant VHH, for 2x Cys conjugation [CTK0101] | srbGCys2-1-500 |
| Nano-Secondary® alpaca anti-rabbit IgG, recombinant VHH, for 2x Cys conjugation [CTK0102] | srbGCys2-2-500 |
| Nano-Secondary® alpaca anti-mouse IgG1, recombinant VHH, for 2x Cys conjugation [CTK0103] | smsG1Cys2-1-500 |
| Nano-Secondary® alpaca anti-mouse IgG1, recombinant VHH, for 2x Cys conjugation [CTK0104] | smsG1Cys2-2-500 |
| Nano-Secondary® alpaca anti-mouse IgG2b, recombinant VHH, for 2x Cys conjugation [CTK0105] | smsG2bCys2-1-500 |
| Nano-Secondary® alpaca anti-mouse IgG2b, recombinant VHH, for 2x Cys conjugation [CTK0106] | smsG2bCys2-2-500 |

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

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