Nano-Secondary® alpaca anti-rabbit IgG, recombinant VHH, for 2x Cys conjugation [CTK0102]

Product code: srbGCys2-2

Properties

Description	Monovalent, recombinant secondary single domain antibody to rabbit IgG: alpaca monoclonal Nanobody, Fab-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	Fab-fragment of rabbit IgG
Cross-reactivity	No cross-reactivity to goat, guinea pig, human, macaque, mouse, rat, and sheep serum
Immunogen	Purified rabbit IgG
Clonality	Monoclonal Nanobody
Conjugate chemistry	N- and C-terminal cysteine conjugation with thiol-reactive reagents, e.g. maleimides
Clone	CTK0102
Molecular weight	15.3 kDa
Extinction coefficient (280 nm)	27,180 M^{-1}*cm^{-1}
Affinity (*Kd*) of unconjugated Nano-Secondary	*Kd* = 1.2 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_2864262

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.

Contact

support@chromotek.com

ChromoTek GmbH
Am Klopferspitz 19
82152 Planegg-Martinsried
Germany
phone: +49 89 124 148 80
fax: +49 89 124 148 811

ChromoTek Inc.
62-64 Enter Lane
Islandia, NY 11749
USA
phone: 631 501 1058
fax: 631 501 1060

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