Nano-Secondary® alpaca anti-rabbit IgG Fc, recombinant VHH, for 2x Cys conjugation [CTK0119]

Product code: srb2GCys2-2

Properties

Description	Monovalent, recombinant secondary single domain antibody to rabbit IgG: alpaca monoclonal Nanobody, Fc-specific, for 2x Cys conjugation
Product type	Nano-Secondary® Reagent, secondary Nanobody (VHH)
Format	Alpaca single domain antibody, monovalent
Host	Alpaca-derived, recombinantly produced in bacteria
Target/Specificity	Fc-fragment of rabbit IgG
Cross-reactivity	No cross-reactivity to mouse, rat or human IgGs
Immunogen	Purified rabbit IgG
Clonality	Monoclonal Nanobody
Conjugate chemistry	N- and C-terminal cysteine conjugation with thiol-reactive reagents, e.g. maleimides
Clone	CTK0119
Molecular weight	14.9 Da
Extinction coefficient (280 nm)	32.680 L * Mol-1 * cm-1
Affinity (*Kd*) of unconjugated Nano-Secondary®	CTK0119: *Kd* = 0.2 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 -80°C. Avoid freeze-thaw cycles. Stable for 1 year at -80°C.
Size	500 µg
RRID	AB_3094643

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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.

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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.

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