Nano-CaptureLigand™ human IgE, VHH, biotinylated

Product code: shuEB-1

Introduction

The ChromoTek Nano-CaptureLigand™ human IgE, VHH, biotinylated is used for the site-directed and specific immobilization of human IgE in biosensor and ELISA assays. It captures non-biotinylated human IgE antibodies to streptavidin/avidin. Nano-CaptureLigand human IgE, VHH, biotinylated comprises a monoclonal biotinylated VHH/ Nanobody. The product belongs to the Nano-CaptureLigands™ family.

Properties

Description	Monovalent, recombinant single domain antibody for the immobilization of human IgE: alpaca monoclonal Nanobody, IgE-specific, biotinylated
Product Type	Capture Nanobody (VHH)
Applications	Immobilization of human IgE antibodies on avidin and streptavidin surfaces for Bio-Layer Interferometry (BLI), Surface Plasmon Resonance (SPR) and ELISA
Target / Specificity	human IgE
Cross-reactivity	No cross-reactivity to human IgG1 (kappa and lambda LC), IgG2, IgG3, IgG4, IgA, IgM; mouse IgE, Ig1 (kappa and lambda LC), IgG2a, IgG2b, IgG2c, IgG3; rabbit IgG
Affinity (*Kd*) of monovalent (1:1) binding mode	0.72 nM Apparent affinity may be higher for full IgE due to avidity effects (1 antibody captured by 2 Nanobodies).
Concentration	1 g/L (68 µM)
Conjugate	Biotin
Degree of biotinylation	On average 1-2 biotin molecules per Nanobody
Format	Alpaca single domain antibody, monovalent
Host	Alpaca-derived, recombinantly produced in bacteria
Clonality	Monoclonal
Clone	CTK0111 (VHH0428)
RRID	AB_2864256
Synonyms	Alpaca single domain antibody, VHH, Nanobody, binding domain of single domain antibody, Nano-antibody

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Validation	Application validated for ELISA and BLI (FortéBio Octet® systems) Determination of cross-reactivity, subclass specificity, sequence, affinity, and melting temperature
Purity	Recombinantly expressed and purified via His-tag
Form	Buffered aqueous solution
Storage buffer	25 mM TAPS pH 8.5, 500 mM NaCl, 5 mM EDTA, Preservative: 0.09 % sodium azide
Storage conditions	Upon receipt store at +4°C/+40°F. *Optional*: Aliquot upon arrival and store at -20°C/-4°F
Stability	Stable for 1 year at +4°C/+40°F
Shipment	Shipped at ambient temperature

Product sizes

Product	Product code	Size
Nano-CaptureLigand™ human IgE, VHH, biotinylated	shuEB-1-10	10 µL
	shuEB-1-100	100 µL

Suggested buffer compositions

Recommended buffers for BLI

Buffer	Composition
1x Kinetics buffer	PBS, 0.01 % (m/v) BSA, 0.002% (v/v) Tween-20
Regeneration buffer	0.01 M glycine, pH 2

Recommended buffers for ELISA

Buffer	Composition
Blocking buffer	PBS, 3 % BSA
Dilution buffer	PBS, 0.05 % Tween-20, 0.5 % BSA
Wash buffer for avidin coating	PBS
Wash buffer	PBS, 0.05 % Tween-20

Bio-Layer Interferometry (BLI) protocol

General notes

- The following protocol was designed for the use with a FortéBio Octet Red96e system. Other BLI systems or your specific research question may require optimization of particular parameters.
- Use the recommended materials or their equivalents.
- Set up all samples in a black 96-well microplate (e.g. Greiner Microplate 96 well, PP, flat-bottom, black, #655209) at room temperature. Use 200 µL per well.
- Nano-CaptureLigands are highly compatible with avidin or streptavidin sensors (e.g. FortéBio Streptavidin (SA) Biosensors, #18-5019) and FortéBio Octet® and and BLItz® systems.
- Run all experiments at +30°C, a shaking speed of 1000 rpm and a recording rate of 5 Hz.
- Dilute all samples in 1x Kinetics buffer. *Optional:* Use 10x Kinetics buffer.
- NanoCaptureLigands can be regenerated at least 10 times with Regeneration buffer with minimal loss of binding efficiency.
- Nano-CaptureLigands carry a His-tag; thus, avoid the use of anti-His primary antibodies.
- Briefly centrifuge the Nano-CaptureLigand solution before use.

Protocol

1. Baseline 1:

- Incubate the biosensors for 60 s in 1x Kinetics buffer.

2. Loading

- Dilute the Nano-CaptureLigand to a concentration of 1 µg/mL in 200 µL 1x Kinetics buffer.
- Load the diluted Nano-CaptureLigand onto the biosensors for 60-120 s until a loading response of 1 nm is reached.

Optional: Use the threshold limit function in the FortéBio Data Acquisition software.

3. Quenching (*optional*)

- Incubate the biosensors for 60 s with biocytin (10 µg/mL in 1x Kinetics buffer) .

4. Baseline 2

- Incubate the biosensors for 120 s in 1x Kinetics buffer.

5. Activation

- Activate the biosensors for 120-180 s with the antibody (20 nM in 1x Kinetics buffer).

6. Baseline 3

- Incubate the biosensors for 120 s in 1x Kinetics buffer.

7. Association

- Bind different antigen concentrations in 1x Kinetics buffer for 120-600 s.

Note: As a start, use 0.1-250 µg/mL or 1/10-10x *Kd* of antigen.

8. Dissociation

- Incubate the biosensors for 60-800 s in 1x Kinetics buffer.

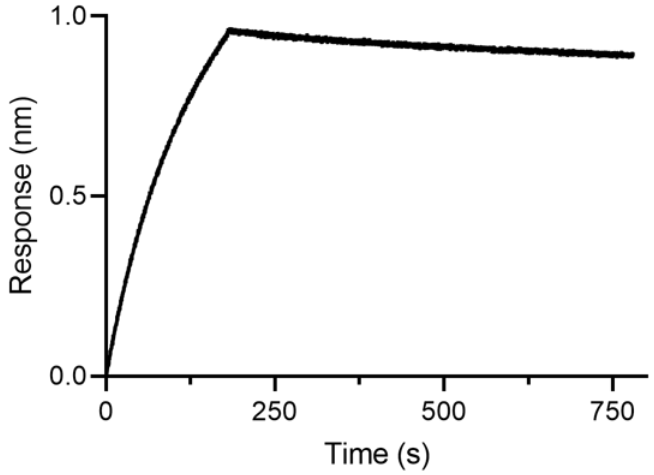
Note: Use the wells from step 6, Baseline 3.

Note: Duration of dissociation step depends on the affinity of the analyzed interaction.

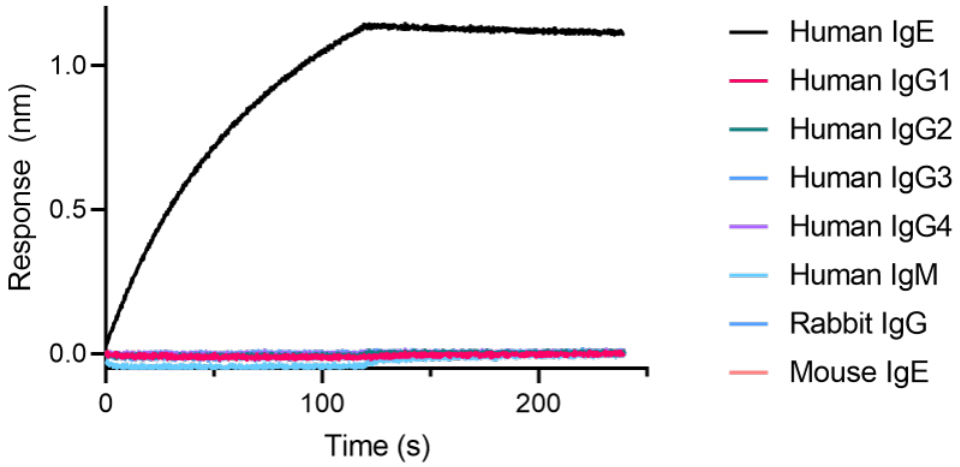
9. Regeneration (*optional*)

- Regenerate the biosensors for 5 s with Regeneration buffer.
- Incubate in 1x Kinetics buffer for neutralization.
- Repeat regeneration 2 times.

Application examples



BLI binding kinetics of a human IgE antibody. 20 nM human IgE antibody was immobilized using Nano-CaptureLigand human IgE, VHH, biotinylated on FortéBio Streptavidin (SA) Biosensors. Human IgE is stably captured by the Nano-CaptureLigand with negligible dissociation.



BLI binding kinetics of human, mouse and rabbit antibodies. 20 nM of human, mouse and rabbit antibodies were immobilized using Nano-CaptureLigand human IgE, VHH, biotinylated on FortéBio Streptavidin (SA) Biosensors. Only human IgE antibody is captured by the Nano-CaptureLigand with negligible dissociation.

Sandwich ELISA protocol

General notes

- The following protocol was designed for a standard sandwich ELISA. Other types of ELISA or your specific research question may require optimization of particular parameters.
- Use the recommended materials or their equivalents.
- In this protocol, MaxiSorp plates (e.g. Thermo Scientific™ White and Black 384-Well Immuno Plates, #460518) are used that must be coated with avidin or streptavidin first. Alternatively, pre-coated avidin/streptavidin plates can be used.
- Nano-CaptureLigands carry a His-tag; thus, avoid the use of anti-His primary antibodies.
- Recommended volumes for 96-well and 384-well microplates:

Protocol steps	96-well microplate	384 -well microplates
Coating, antigen binding, antibody binding	100 µL	20 µL
Washing, blocking	300 µL	90 µL

- Briefly centrifuge the Nano-CaptureLigand solution before use.

Protocol

1. Avidin coating (*optional*)

- Coat each well of a MaxiSorp plate with 10 µg/mL avidin in PBS at +4°C overnight.
- Wash each well twice with PBS.

2. Blocking

- Block each well with Blocking buffer for 1-2 h at room temperature.
- Wash each well 3 times with Wash buffer.

3. Nano-CaptureLigand coating

- Add 50 nM Nano-CaptureLigand (diluted in Dilution buffer) to each well.
- Incubate for 1 h at room temperature.
- Wash each well 5 times with Wash buffer.

4. Immobilization of capture antibody

- Add the capture antibody (diluted in Dilution buffer) to each well.
- Incubate for 1 h at room temperature.
- Wash each well 5 times with Wash buffer.

Note: Test different concentrations of the capture antibody in an initial experiment.

5. Antigen binding

- Add the antigen to each well.
- Incubate for 1 h at room temperature.
- Wash each well 5 times with Wash buffer.

Note: Test different concentrations of the antigen.

6. Binding of primary antibody

- Add the primary antibody to each well and incubate.
- Wash each well 5 times with Wash buffer.

Note: Dilute and incubate the primary antibody as indicated in the manufacturer's manual.

7. Binding of secondary / detection antibody

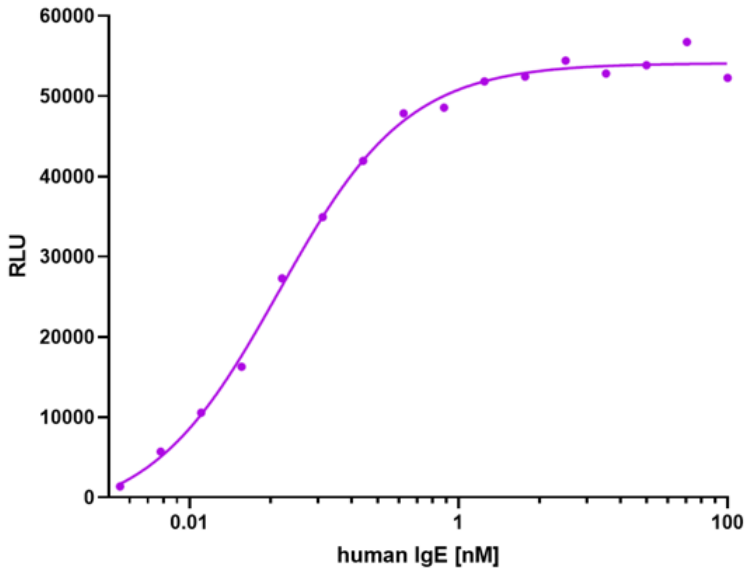
- Add the secondary / detection antibody to each well and incubate.
- Wash each well 5 times with Wash buffer.

Note: Dilute and incubate the secondary / detection antibody as indicated in the manufacturer's manual.

8. Detection

- Add the appropriate ELISA substrate solution to each well and incubate as indicated in the manufacturer's manual.
- Analyze with a microplate reader.

Application examples



ELISA capture of a human IgE antibody using Nano-CaptureLigand human IgE, VHH, biotinylated. 50 nM Nano-CaptureLigand human IgE, VHH, biotinylated was used for coating on an avidin-coated MaxiSorp plate. Human IgE antibody was titrated in a 1:2 dilution series and detected with a rabbit anti-human IgE primary antibody and an alkaline phosphatase-conjugated detection antibody.

Product overview and related products

Product	Product code
Nano-CaptureLigand™ human IgG/rabbit IgG, Fc-specific VHH, biotinylated	shurbGB-1-10; -100
Nano-CaptureLigand™ human Ig, lambda-LC-specific VHH, biotinylated	shuLB-1-10; -100
Nano-CaptureLigand™ human IgE, VHH, biotinylated	shuEB-1-10; -100
Nano-CaptureLigand™ mouse IgG1, Fc-specific VHH, biotinylated	smsG1B-1-10; -100
Nano-CaptureLigand™ mouse IgG2a, Fc-specific VHH, biotinylated	smsG2aB-1-10; -100
Nano-CaptureLigand™ mouse IgG2b, Fc-specific VHH, biotinylated	smsG2bB-1-10; -100
Nano-CaptureLigand™ mouse IgE, VHH, biotinylated	smsEB-1-10; -100
GFP VHH, biotinylated recombinant binding protein	gtb-250

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

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