DIYEK C1

Combined human Endocan and Cathepsin G-cleaved Endocan

References: LIK-1501, LIK-1501B5, LIK-1501B10, LIK-1501B25

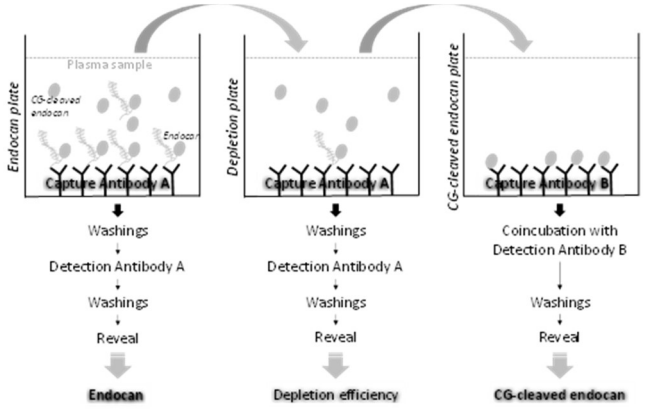
The combined human endocan and CG (Cathepsin G)- cleaved endocan DIY (Do It Yourself) ELISA kit (DIYEK) contains the key components required for quantification of both human endocan and its proteolytic product called CG-cleaved endocan in cell culture supernatants, serum, or plasma. The components supplied in this kit are sufficient to perform two, five, ten or twenty-five 96-well plates for endocan and CG-cleaved endocan ELISA.

PRINCIPLE OF THE METHOD

Cathepsin G-cleaved endocan represents the 111-116 N-terminal amino acids of endocan, losing the 49-54 C-terminal amino acids (endocan C-Ter peptide). Cathepsin G-cleaved endocan has been found circulating together with endocan in blood from patients with severe infection or sepsis.

To quantify CG-cleaved endocan, previous elimination of endocan is required. The Capture Antibody A, which maps the endocan C-Ter peptide, and routinely used for measuring endocan, gives advantage to be also used for depleting endocan.

The method here described, combines the advantages of Capture Antibody A-coated plates for both measuring and depleting endocan. Full endocan depletion includes an additional incubation with Capture Antibody A-coated plates (which optionally quantify the residual endocan after a first depletion).



SOLUTIONS AND MATERIAL REQUIRED - NOT INCLUDED

Buffer A: Carbonate/Bicarbonate buffer 0.1 M, pH 9.6 (Biothelis, ref. LIM-1210)
Buffer B: PBS containing 0.1% BSA, 5 mM EDTA, 0.1% Tween 20 (Biothelis, ref. LIM-1201)
Substrate solution: TMB Substrate Solution (Biothelis, ref. LIM-1207)
Stop solution: 2N H_2SO_4 (Biothelis, ref. LIM-1209)
Enzyme reagent: Streptavidin-HRP (Biothelis, ref. LIM-1203)
Tubes: Polypropylene tubes for dilution
Microplates: 96-well ELISA plates
Shaker: Horizontal orbital microplate shaker
Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set to 630 nm

COMPONENTS

Capture Antibody A – The vial contains capture antibody at 5 mg/mL.
Capture Antibody B – The vial contains capture antibody at 5 mg/mL.
Lyophilized Human Endocan Standard – Lyophilized recombinant Human Endocan. After reconstitution, aliquot and store Endocan Standard at -70°C for up to 6 months. The volume of reconstitution is indicated on the vial.
Detection Antibody A – The vial contains biotinylated detection antibody at 2 mg/mL.
Detection Antibody B – The vial contains biotinylated detection antibody at 2 mg/mL.

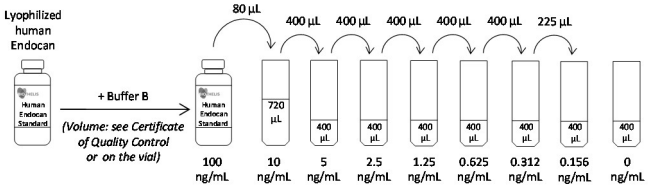
	LIK-1501	LIK-1501B5	LIK-1501B10	LIK-1501B25
Capture Antibody A	70 µL	175 µL	340 µL	800 µL
Capture Antibody B	210 µL	525 µL	1050 µL	2x1275 µL
Detection Antibody A	10 µL	15 µL	25 µL	55 µL
Detection Antibody B	10 µL	15 µL	25 µL	55 µL
Standard Endocan	1 vial	2vials	4 vials	10 vials

STORAGE INFORMATION

Store unopened reagents at +4°C.

STANDARD PREPARATION FOR ASSAY

1. After warming lyophilized standard to room temperature (RT), carefully open vial to avoid any loss of material. Reconstitute lyophilized Human Endocan Standard with the volume of Buffer B indicated directly on the vial, to obtain a solution at 100 ng/mL. After reconstitution, Standard solution should be aliquoted and stored at -70°C for next use.
2. Prepare the high standard (10 ng/mL) from the reconstituted standard solution. We recommend pipetting 80 µL of the reconstituted standard solution into 720 µL of Buffer B.
3. Add 400 µL Buffer B to 6 tubes (always use polypropylene tubes).
4. Perform serial dilutions by adding 400 µL of each standard (2-fold dilution) to the next tube and mix each tube thoroughly between each dilution. Buffer B serves as the blank.



PREPARATION OF ENDOCAN, DEPLETION, and CG-CLEAVED ENDOCAN PLATES

Before use, bring all reagents to RT i.e 18-25°C. We recommended that samples, standards and controls be assayed in duplicate.
1. Dilute the Capture Antibody A to a working concentration of 7.5 µg/mL in Buffer A and coat two 96-well microplates with 100 µL per well of the diluted Capture Antibody A. These microplates are called ***endocan plate*** for measuring endocan, and ***depletion plate***. Seal / cover the plate and incubate overnight at +4°C.

Dilute the Capture Antibody B to a working concentration of 50 µg/mL in Buffer A and coat a 96-well microplate with 100 µL per well of the diluted Capture Antibody B. This plate is called ***CG-cleaved endocan plate***, for detecting CG-cleaved endocan. Seal / cover the plate and incubate overnight at +4°C.
2. Remove the Capture Antibody A and B by inverting the plates and wash each well three times with 300 µL of Buffer B. After the last wash, remove any remaining buffer by inverting the plate and blotting it against clean paper towels.
4. Add 300 µL of Buffer B to each well for 1h at RT to block plates.
5. Wash three times each well with 300 µL of Buffer B.
6 Add 100 µL of Buffer B and keep the plates at room temperature until use.

SANDWICH ELISA PROTOCOL FOR ENDOCAN

1. Add 100 µL of human endocan Standards and Samples (diluted or not) into the ***endocan plate***. Cover the plate and incubate for 1h at RT with gentle agitation on orbital shaker.
2. Transfer the samples from the ***endocan plate to the depletion plate*** previously discharged of Buffer B (cf ENDOCAN DEPLETION paragraph).
3. Wash three times the ***endocan plate*** with 300 µL of Buffer B.
4. Add 100 µL of Detection Antibody A, diluted 10 000 fold in Buffer B. Cover the plate and incubate for 1h at RT with gentle agitation.
5. Wash three times each well with 300 µL of Buffer B.
6. Add 100 µL of Streptavidin-HRP diluted in Buffer B. Cover the plate and incubate for 30 min at RT with gentle agitation. Protect from light.
7. Wash three times each well with 300 µL of buffer B.
8. Add 100 µL of Substrate Solution to each well and incubate at RT until a blue byproduct is observed. Protect from light.
9. Add 100 µL of Stop Solution to each well.
10. Determine the optical density using a microplate reader set to 450 nm, with wavelength correction set to 630 nm.

ENDOCAN DEPLETION

11. Just after the transfer of the sample wells from ***endocan-*** to the ***depletion- plate*** **in step 2 (**following an identical plate scheme) add 100 µL of the human Endocan standards Cover the plate and incubate for 1h at RT with gentle agitation on orbital shaker.
12. Transfer the samples from the ***depletion plate*** to the ***CG-cleaved endocan plate***, previously discharged of buffer B.
13. Repeat the steps 3-10 on the ***depletion plate*** for quantifying the efficiency of the depletion (optional).

SANDWICH ELISA PROTOCOL FOR CG-CLEAVED ENDOCAN

14. Just after the transfer of the sample wells from the ***depletion-*** to the ***CG-cleaved endocan- plate, in step 12,*** add 100 µL of human endocan standards into the ***CG-cleaved endocan plate***. Then, add 10 µL of the Detection Antibody B, diluted 500 fold in Buffer B, to each well. Cover the plate and incubate for 2h at RT with gentle agitation on orbital shaker.
15. Apply the steps 5-10 to the ***CG-cleaved endocan plate***.

SYNOPSIS

Plate's name :	Endocan	Depletion	CG-cleaved endocan
Coating antibody	A	A	B
Detection antibody	A	A	B
Sample incubation time	1 hour	1 hour	2 hours
T0	Endocan saturation	Endocan saturation	Endocan saturation
T+1h	Endocan immobilisation	Incubation in washing buffer	Incubation in washing buffer
T+2h	Endocan detection	Endocan immobilisation	
T+3h	Revelation	Endocan detection	Endocan immobilisation and detection
T+4h		Revelation	
T+5h			Revelation

SPECIFICITY

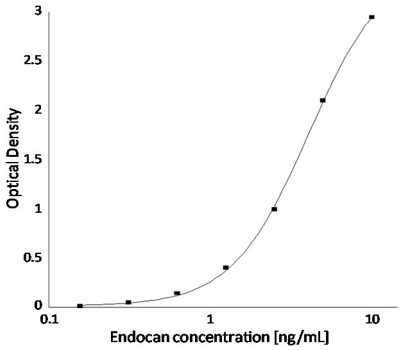
Cross reactivity - No cross reactivity was observed with mouse or rat endocan at 10 ng/mL when assayed in the sandwich ELISA assay.
Matrix compatibility - Cell culture supernatant, cell lysates, serum or plasma with optimal dilutions ranging from 1:2 to 1:8 in buffer B.
High sensitivity - 0,5-10 ng/ml for extended protocol.
Spike recovery - 97% ± 6% in serum, and 94% ± 6% in plasma EDTA (mean ± SD)[3].

CALCULATION OF RESULTS

Substract the zero standard optical density to the optical density of each standard and samples.
Create a standard curve by reducing the data using a computer software generating a lin-log four parameter curve-fit. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL STANDARD CURVE

The standard curve below is only for demonstration purposes. A standard curve should be generated for each set of samples assayed. The same endocan standard is used to quantify CG-cleaved endocan, but calibrated in a different pair of antibodies (Capture/Detection Antibody B).



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4. **Zafrani L, et al. (2018)** Endothelial Cell-Specific Molecule-1 in Critically Ill Patients With Hematologic Malignancy, Crit Care Med. 46(3):e250-e257
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6. **Gaudet et al. (2020)** Cleaved endocan acts as a biologic competitor of endocan in the control of ICAM-1-dependent leukocyte diapedesis, J Leukoc Biol, 107(5):833-841.

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