DIYEK R1
Rat Endocan / ESM-1 ELISA Kit
Reference: LIK-1103

Rat Endocan / ESM-1 "Do It Yourself" ELISA Kit H1 (DIYEK R1) contains the key components required for quantification of m Endocan in cell culture supernatants, serum, or plasma. The components provided in this kit are sufficient to realize two, five, ten or twenty-five 96-well plates.

REAGENTS PROVIDED AND STORAGE CONDITIONS

Store unopened reagents at +4°C.

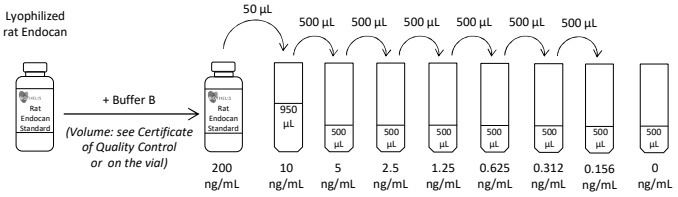
Capture Antibody – The vial contains 110 µL of capture antibody at 1 mg/mL. Store at +4°C.
Detection Antibody - The vial contains 10 µL of biotinylated detection antibody. Store at +4°C.
Lyophilized Rat Endocan Standard – One vial of recombinant endocan. After reconstitution, aliquot and store Endocan Standard at -70°C for up to 6 months. The volume of reconstitution is indicated on the vial.

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)
Buffer C : PBS containing 0.1% BSA, 5 mM EDTA, 0.1% Tween 20, 2.5 M NaCl (Biothelis ref. LIM-1202)
Substrate solution : TMB Substrate Solution (Biothelis ref. LIM-1207)
Stop solution : 2N H_2SO_4 or 4N HCl (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

STANDARD PREPARATION FOR ASSAY

1. After warming lyophilized standard to room temperature (RT), carefully open vial to avoid any loss of material. Reconstitute lyophilized Rat Endocan Standard with the volume of Buffer B indicated in the Certificate of Quality Control and directly on the vial, to obtain a solution at 200 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 50 µL of the reconstituted standard solution into 950 µL of Buffer B.
3. Add 500 µL Buffer B to 6 tubes (always use polypropylene tubes).
4. Perform serial dilutions by adding 500 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.



SAMPLE DILUTION FOR ASSAY

Use polypropylene tubes and Buffer B for sample dilutions.
Serum and plasma : do not dilute the sample.
Cell culture supernatant : samples may require dilution according experiment settings.

SANDWICH ELISA PROTOCOL

Before use, bring all reagents to RT i.e 18-25°C. Immediately after use, return to proper storage conditions. We recommend that Samples, Standards and Controls should be assayed in duplicate.
1. Dilute the Capture Antibody to a working concentration of 5 µg/mL in Buffer A and coat a 96-well microplate with 100 µL per well of the diluted Capture Antibody. Seal/cover the plate and incubate overnight at +4°C.
2. Remove the Capture Antibody by inverting the plate 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.
3. Add 300 µL of Buffer B to each well for 1 h at RT to block plates.
4. Wash three times each well with 300 µL of Buffer B as in step 2.
5. Add 100 µL of rat endocan (Standards and Samples, diluted or not). Then add 10 µL of the Detection Antibody, diluted 500 fold in Buffer C, to each well. Cover the plate and incubate with gentle agitation (400 rpm):
   - 2 h at RT for cell culture supernatant samples
   - overnight at 4-6°C for serum or plasma samples
6. Wash three times each well with 300 µL of Buffer B as in step 2.
7. 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.
8. Wash four times each well with 300 µL of buffer B as in step 2.
9. Add 100 µL of Substrate Solution to each well and incubate at RT until a blue byproduct is observed. Protect from light.
12. Add 100 µL of Stop Solution to each well.
13. Determine the optical density using a microplate reader set to 450 nm, with wavelength correction set to 630 nm.

SPECIFICITY

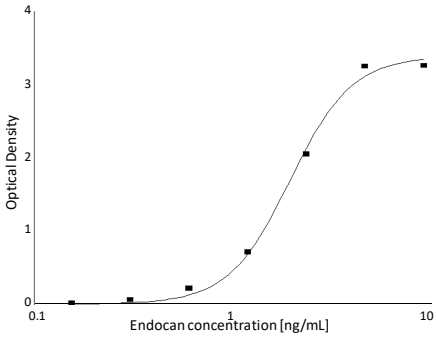
A cross reactivity was observed between mouse and rat endocan, but not with human endocan, when assayed in the sandwich ELISA assay.

CALCULATION OF RESULTS

Substract the blank 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.



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