Rat Endocan / ESM-1
DIY ELISA Kit EndoMark R1

Reference: LIK-1103

Rat Endocan / ESM-1 DIY «Do It Yourself» ELISA kit EndoMark R1 (DIYEK R1) contains the key components required for quantification of rat Endocan / ESM-1 in cell culture supernatants, serum, or plasma. The components provided in this kit are sufficient to realize two 96-well plates.

REAGENTS PROVIDED

Capture Antibody – The vial contains 100 µL of capture antibody (1 mg/mL).
Lyophilized Endocan Standard – Two vials of recombinant Endocan / ESM-1 per vial (enough to perform a seven point standard curve ranging from 10 to 0.15 ng/mL).
Detection Antibody – The vial contains 10 µL of biotinylated antibody for detection of rat Endocan.

SOLUTIONS AND MATERIAL REQUIRED – NOT INCLUDED

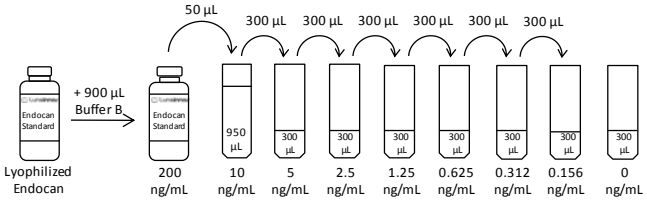
Buffer A: Carbonate/Bicarbonate buffer 0.1 M, pH 9.6
Buffer B: PBS containing 0.1% BSA, 5 mM EDTA, 0.1% Tween 20
Buffer C : PBS containing 0.1% BSA, 5 mM EDTA, 0.1% Tween 20, 2.5 M NaCl
Substrate solution: TMB Substrate Solution (recommended: Interchim, ref: UP664781)
Stop solution: 2N H_2SO_4 or 4N HCl
Enzyme reagent: Streptavidin-HRP (Invitrogen, ref: 43-4323)
Tubes : Polypropylene tubes for dilution
Microplates : 96-well ELISA plates (recommended: Maxisorp Nunc-immunoplate, ref: 430341)
Shaker : Horizontal orbital microplate shaker
Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set to 630 nm

STORAGE INFORMATION

Store unopened reagents at -20°C.

STANDARD PREPARATION FOR ASSAY

1. After warming of the Lyophilized Standard at room temperature (RT), carefully open the vial to avoid any loss of material. Then, reconstitute Lyophilized Endocan Standard with 900 µL of Buffer B to obtain a solution at 200 ng/mL.
2. After reconstitution, never leave reconstituted Standard at RT but keep it on ice. Standard solution should never be frozen for the next use.
3. Prepare the highest concentration of 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.
4. Add 300 µL Buffer B to 6 tubes (always use polypropylene tubes).
5. Perform serial dilutions by adding 300 µL of each Standard (2-fold dilution) to the next tube and mix each tube thoroughly between each dilution.
Buffer B serves as the zero standard (0 ng/mL).



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 to experiment settings.

SANDWICH ELISA PROTOCOL

Before to 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 washing 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 and wait for 1 h at RT to block plate.
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 10 000 fold in Buffer B (we recommend that you should always titrate a new batch of Streptavidin-HRP). 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 for 10 to 20 minutes at RT until a blue byproduct is observed. Keep away from light.
10. Add 50 µL of Stop Solution to each well.
11. Determine the optical density using a microplate reader set to 450 nm, with wavelength correction set to 630 nm.

SPECIFICITY

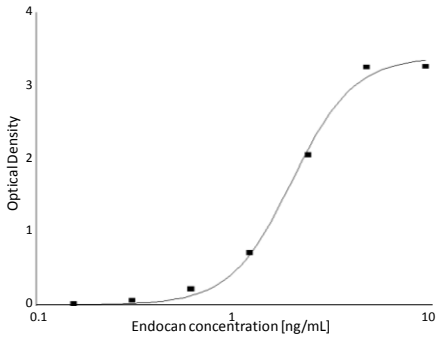
A cross reactivity was observed between mouse and rat endocan, but not with human endocan, when assayed in the sandwich ELISA assay.

CALCULATION OF RESULTS

Substract the zero standard optical density to the optical density of each Standard and Sample.
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.



REFERENCES

Bechard et al. (2000) Characterization of the secreted form of ESM-1 by specific monoclonal antibodies. J. Vasc. Res. 37:417-425.
Depontieu et al. (2012) Development of monoclonal antibodies and ELISA specific for the mouse vascular endocan. J Immunol Methods. 378:88-94.
Grigoriu et al. (2006) Endocan expression and relationship with survival in human non-small cell lung cancer. Clin. Cancer Res. 12:4575-4582.
Pastré et al. (2010) Differential kinetics of endothelial cell activation biomarkers E-selectin and Endocan during nonlethal endotoxemia in 129Sv mice: a role for PMN-derived serine proteases in the transient decrease of circulating endocan levels. Crit. Care 14 (Suppl 2):P42.
Scherpereel et al. (2006) Endocan, a new endothelial marker in human sepsis. Crit. Care Med. 34:532-537.
Tissier et al. (2004) Calpain inhibitors improve myocardial dysfunction and inflammation induced by endotoxin in rats. Shock. 21:352-357.

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NOT FOR USE IN DIAGNOSTIC PROCEDURES

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