

**DIYEK H1****Human Endocan / ESM-1 ELISA Kit**

References: LIK-1101, LIK-1101B5, LIK-1101B10, LIK-1101B25

Human Endocan / ESM-1 “Do It Yourself” ELISA Kit H1 (DIYEK H1) contains the key components required for quantification of human 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** – One vial with mouse anti-human endocan antibody at 1 mg/mL. Store at +4°C.

**Detection Antibody** – One vial with biotinylated mouse anti-human endocan antibody at 2 mg/mL. Store at +4°C.

**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 in the Certificate of Quality Control and on the vial.

Reference	LIK-1101	LIK-1101B5	LIK-1101B10	LIK-1101B25
<b>Capture Antibody</b>	50 µL	120 µL	240 µL	600 µL
<b>Detection Antibody</b>	10 µL	10 µL	12 µL	30 µL
<b>Endocan Standard</b>	1 vial	1 vial	2 vials	5 vials

**SOLUTIONS AND MATERIAL REQUIRED - NOT INCLUDED**

**Buffer A:** Carbonate/Bicarbonate buffer 0.1 M, pH 9.6 (Biothelisis ref. LIM-1210)

**Buffer B:** PBS containing 0.1% BSA, 5 mM EDTA, 0.1% Tween 20 (Biothelisis ref. LIM-1201)

**Substrate solution:** TMB Substrate Solution (Biothelisis ref. LIM-1207)

**Stop solution:** 2N H<sub>2</sub>SO<sub>4</sub> (Biothelisis ref. LIM-1209)

**Enzyme reagent:** Streptavidin-HRP (Biothelisis ref. LIM-1203)

**Tubes:** Polypropylene tubes for dilution

**Microplates:** 96-well ELISA plate

**Shaker:** Horizontal orbital microplate shaker

**Microplate reader** capable of measuring absorbance at 450 nm, with the correction wavelength set to 630 nm

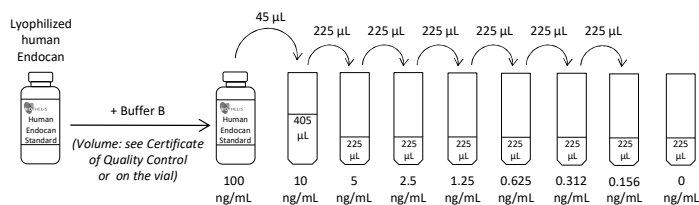
**STANDARD PREPARATION FOR ASSAY**

1. After warming lyophilized standard to room temperature (RT), carefully open vial to avoid any loss of material. Reconstitute lyophilized Human 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 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 45 µL of the reconstituted standard solution into 405 µL of Buffer B.

3. Add 225 µL Buffer B to 6 tubes (always use polypropylene tubes).

4. Perform serial dilutions by adding 225 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:** may require a dilution ranging from 1:2 to 1:10 according clinical context.

**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. We recommended that samples, standards and blank should be assayed in duplicate.

1. Dilute the Capture Antibody to a working concentration of 2 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 human endocan (Standards and Samples, diluted or not). Cover the plate and incubate for 1 h at RT with gentle agitation.

6. Wash three times each well with 300 µL of Buffer B as in step 2.

7. Add 100 µL of Detection Antibody, diluted 10 000 fold in Buffer B. Cover the plate and incubate for 1 h at RT with gentle agitation.

8. Wash three times each well with 300 µL of Buffer B as in step 2.

9. Add 100 µL of Streptavidin-HRP, diluted 1000 fold in Buffer B. Cover the plate and incubate for 30 min at RT with gentle agitation. Protect from light.

10. Wash three times each well with 300 µL of buffer B as in step 2.

11. Add 100 µL of Substrate Solution to each well and incubate for 10 minutes 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**

No cross reactivity was observed with mouse or rat endocan at 10 ng/mL when assayed in the sandwich ELISA assay.

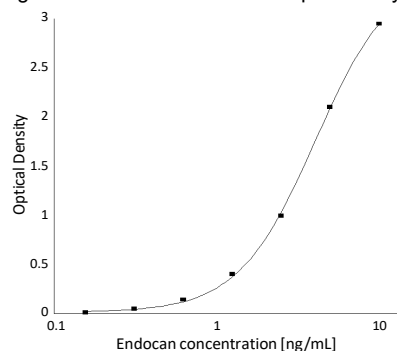
**CALCULATION OF RESULTS**

Subtract 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.

**REFERENCES**

**Gaudet et al. (2019)** Endocan regulates acute lung inflammation through control of leukocyte diapedesis. *J Appl Physiol Bethesda Md* 1985 127:668–678.

**Gaudet et al. (2018)** Low endocan levels are predictive of Acute Respiratory Distress Syndrome in severe sepsis and septic shock. *J Crit Care* 47:121–126.

**De Freitas Caires et al. (2018)** Endocan, sepsis, pneumonia, and acute respiratory distress syndrome. *Crit Care Lond Engl* 22:280.

**Palud et al. (2015)** Evaluation of endothelial biomarkers as predictors of organ failures in septic shock patients. *Cytokine*. 2015 Jun;73(2):213-8.

**Mikkelsen et al. (2012)** Lower serum endocan levels are associated with the development of acute lung injury after major trauma. *J Crit Care* 27(5):522.

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NOT FOR USE IN DIAGNOSTIC PROCEDURES