JDIEK H1

“Just Do It ELISA Kit H1”

ELISA kit to detect and quantify human Endocan / ESM-1 in cell culture supernatants, serum and plasma.

Reference: LIK-1205

NOT FOR USE IN DIAGNOSTIC PROCEDURES
FOR RESEARCH USE ONLY

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BACKGROUND

What is endocan?

Endocan, also known as endothelial cell-specific molecule 1 (ESM-1), was originally discovered in endothelial cells[1]. The preferred expression in the lung is governed by the proximal promoter region[2]. Endocan is a 50 kDa proteoglycan constituted of a 165 aminoacid mature protein core (20 kDa), and a unique chondroitin/dermatan sulfate chain linked to the serine residue at position 137[3, 4].

What does endocan do?

Endocan is co-mitogenic through its glycan chain by inducing cell migration, proliferation and tumor growth[5–10]. Endocan exhibits also anti-inflammatory properties by inhibiting the leukocyte adhesion through endothelial cells[11–14].

How endocan is regulated?

There is a spontaneous synthesis and secretion by endothelial cells. This expression can be increased by the proinflammatory cytokines TNF-α or IL-1β[15], by bacterial lipopolysaccharide[16], or by the angiogenic factors FGF-2 or VEGF[5, 17, 18]. Instead, this expression can be reduced by IFN-γ[15], or angiopoietin-1 via the transcription factor FOXO1[19, 20].

Clinical studies

Its elective pulmonary endothelial expression has led to establish a significant relationship between the blood endocan levels and the severity of various respiratory diseases (see[21] for a general review), such as community-acquired pneumonia[22–25], post-operative pneumonia[26–28], pneumonia acquired with mechanical ventilation[29, 30], acute respiratory distress syndrome[31–36], and sleep apnea[37–39]

PRINCIPLE OF THE SANDWICH ELISA

This JDIEK H1 assay uses the robust and well-described quantitative sandwich enzyme immunoassay technique. Briefly, a monoclonal antibody specific for human Endocan / ESM-1 (also called Capture Antibody) has been coated onto a 96-well microplate. Samples, like serum, plasma, vitreous humor, or cell culture supernatants, are pipetted into the wells and any Endocan present within the sample is bound by the Capture Antibody (Standards are processed the same way for quantification purpose). After washing away of any unbound molecules, a secondary monoclonal antibody specific for Endocan that has been biotinylated, is added to the wells. After a washing step, a substrate solution is added to each well and color should develop in proportion to the amount of Endocan present in the Samples. The color development is stopped by acid solution and the intensity of the color is measured by spectrophotometry.

RESTRICTIONS / LIMITATIONS OF THE JDIEK H1

- Please read carefully and completely this notice before use.
- Always wear eye, hand, face, and clothing protection when using the Acid Stop Solution.
- Do not substitute reagents with those from other sources / origins.
- Do not mix reagents with those from other sources / origins.
- Do not eat reagents or mix them with food.
- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

TECHNICAL HINTS

- Do not freeze reconstituted standard.
- Always use polypropylene tubes for serial dilution.
- Always avoid foaming when reconstituting standards and when mixing solutions.
- If samples generate values higher than the highest standard, dilute the samples with 1X ELISA Buffer and repeat the assay.
- Change pipette tips to avoid cross-contamination between standard, samples and reagents addition.
- Always use separate reservoirs for each reagent.
- Variations in results can be due from: variations in pipetting or variations in washing techniques; variations in the incubation time; variations in room temperature.
- Avoid repeated freeze-thaw cycles of your samples / biological fluids.
- Always keep TMB solution protected from light.
- Always add the Stop Solution in the same order as the TMB solution.

REAGENTS PROVIDED

ELISA Microplate (Ref. LIM-1208) - One 96 well microplate (6 strips of 16 wells) pre-coated with the Capture Antibody.

Human Endocan standard (Ref. LIP-1101) - Two vials of lyophilized recombinant human Endocan.

Detection Antibody – One bottle with 12 mL of biotinylated monoclonal antibody against Endocan (ready-to-use).

Streptavidin-HRP (Ref. LIM-1302) – One bottle with 12 mL of Streptavidin-HRP (ready-to-use).

ELISA Buffer 20X (Ref. LIM-1206) – One bottle with 75 mL of a 20-fold concentrated solution.

TMB (Ref. LIM-1207) – One bottle with 12 mL of 3,3',5,5'-tétraméthylbenzidine substrate (ready-to-use).

Stop Solution (Ref. LIM-1209) - One bottle with 12 mL of 2N sulfuric acid.

MATERIAL REQUIRED AND NOT INCLUDED

- Horizontal orbital microplate shaker.
- Microplate reader capable of measuring absorbance at 450 nm and with the correction wavelength set to 630 nm.
- Polypropylene tubes for dilution.
- Ultrapure water.
- Pipettes and pipettes tips.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Reservoirs.
- Ice bucket.

SAMPLE COLLECTION AND STORAGE

Plasma – Collect blood in tube with EDTA as anticoagulant. Centrifuge at 1500 x g for 10 minutes at 4°C. Remove plasma and aliquot before storage at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum – Collect blood in tube without anticoagulant. After clot formation, centrifuge at 1500 x g for 10 minutes at 4°C. Remove serum and aliquot before storage at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatant – Remove cellular debris by centrifugation and aliquot the supernatant before storage at ≤ -20°C. Avoid repeated freeze-thaw cycles.

BUFFER AND STANDARD PREPARATION FOR ASSAY

1. Prepare ELISA Buffer (1X) by a 20-fold dilution of concentrate ELISA Buffer (20X): 20 mL of ELISA Buffer (20X) + 380 mL of ultrapure water.

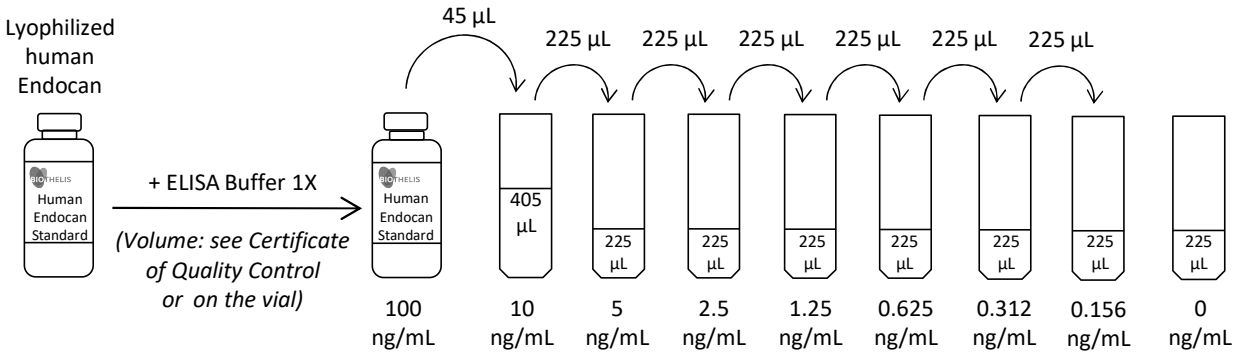
2. After warming of the lyophilized Human Endocan Standard at room temperature (RT), carefully open the vial to avoid any loss of material. Then reconstitute each vial of lyophilized Human Endocan Standard with the volume of ELISA Buffer (1X) indicated in the Certificate of Quality Control and directly on the vial, to obtain a solution at 100 ng/mL.

3. After reconstitution, standard solution should never be frozen.

4. Prepare the highest concentration of Standard (10 ng/mL) from the reconstituted Human Endocan Standard solution. We recommend pipetting 45 µL of the reconstituted Standard solution into 405 µL of ELISA Buffer (1X).

5. Add 225 µL of ELISA Buffer (1X) to 6 tubes (always use polypropylene tubes).

6. 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. ELISA Buffer (1X) serves as the zero standard (0 ng/mL).



SAMPLE DILUTION FOR ASSAY

- Use polypropylene tubes for sample dilution.
- Dilute samples in ELISA Buffer (1X).
- Serum and plasma samples: may require a dilution ranging from 1:2 to 1:10.
- Cell culture supernatants samples: may require dilution according to experiment settings.

SANDWICH ELISA PROTOCOL

Before use, bring all reagents to RT (i.e. 18-25°C). Immediately after use, return to 2-8°C storage temperature. We recommend that Samples, Standards and Controls should be assayed in duplicate.

1. Add 100 µL of Human Endocan (**Standard and Samples**, diluted or not). Cover the plate with an adhesive strip and incubate for 1 h at RT with gentle agitation (450 rpm).

2. Wash three times each well with 250 µL of **ELISA Buffer** (1X).

3. Add 100 µL of **Detection Antibody** (ready-to-use). Cover the plate with an adhesive strip and incubate for 1 h at RT with gentle agitation (450 rpm).

4. Wash three times each well with 250 µL of **ELISA Buffer** as in step 2.

5. Add 100 µL of **Streptavidin-HRP** (ready-to-use). Cover the plate with an ELISA strip and incubate for 30 min at RT with gentle agitation (450 rpm). Keep away from light.

6. Wash three times each well with 250 µL of **ELISA Buffer** 1X as in step 2.

7. Add 100 µL of **TMB** to each well and incubate for 4-10 min at RT until a blue byproduct is observed. Keep away from light.

8. Add 100 µL of **Stop Solution** to each well. The color in the wells will turn from blue to yellow upon addition.

9. Determine the Optical Density (OD) using a microplate reader set to 450 nm and with wavelength correction set to 630 nm.

PROTOCOL SUMMARY

Prepare ELISA Buffer (1X), samples and Standard as recommended

Add 100 µL of Standard and samples to each well.
Incubate 1 hour.

Wash 3 times

Add 100 µL of Detection Antibody.
Incubate 1 hour.

Wash 3 times

Add 100 µL of Streptavidin-HRP.
Incubate 30 minutes. Protect from light.

Wash 3 times

Add 100 µL of TMB.
Incubate 10 minutes. Protect from light.

Add 100 µL of Stop Solution.

Read at 450 nm.
Wavelength correction set to 630 nm.

CALCULATION OF RESULTS

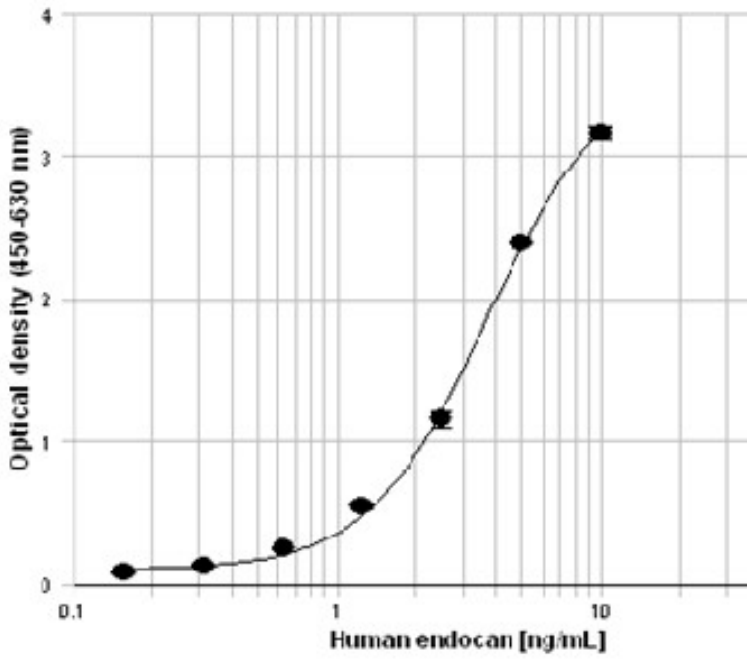
Subtract the zero standard optical density to the optical density of each Standard and each Sample.

Create a standard curve using computer software generating a lin-log four parameter curve-fit. If the samples were diluted, the concentration read from the standard curve should be then multiplied by the dilution factor.

EXAMPLE OF TYPICAL STANDARD CURVE

The standard curve below is only for demonstration purposes.

A standard curve should be generated for each set of Samples assayed.



Limit of quantification	0.3 ng/mL
Detection	0.3 ng/mL to 10 ng/mL
Precision (CV%)	Intra-assay precision : 4.40%
	Inter-assay precision : 7.59%
Interference	No interference was observed with haemolysed or hyperlipidaemic plasma

EXAMPLES OF SAMPLE VALUES IN HUMAN PLASMA AND SERUM

Endocan was measured in plasma and serum from healthy volunteers by us and others (published data). The results are shown in the table below. The values are done in ng/mL.

Sample Type	n	Mean	Extreme	Std. Deviation	Origin	Ref.
Plasma	n=32	1.00	0.40 – 1.80	0.33	France	Biothelis
Serum	n=32	1.18	0.40 – 2.90	0.46	France	Biothelis
Serum	n=20	0.77		0.44	France	11
Serum	n=25		0.12 – 0.31		Turkey	24
Serum	n=35		0.75 – 0.16		Turkey	25
Plasma	n=82	0.91		0.38	Taiwan	23
Serum	n=25	0.63		0.06	France	5

SPECIFICITY

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

There is a 100 % homology between human and monkey Endocan. Therefore this assay may be used to analyze Endocan in biological fluids from monkey origin.

STORAGE INFORMATION

<table>
<tr><td>Unopened Kit</td><td colspan="2">Upon receipt, store all the kit at 2-8°C.
DO NOT USE THE COMPONENTS BEYOND THE EXPIRATION DATE INDICATED ON THE KIT LABEL.</td></tr>
<tr><td rowspan="7">Opened / Reconstituted reagents</td><td>ELISA Buffer (20X)</td><td rowspan="5">May be stored up to 1 month at 2-8°C.*</td></tr>
<tr><td>Detection antibody</td></tr>
<tr><td>Streptavidin-HRP</td></tr>
<tr><td>TMB</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Human Endocan Standard</td><td>Once reconstituted the standard is stable for 6 hours, if stored at 2-8°C. Do not freeze.</td></tr>
<tr><td>ELISA Microplate</td><td>Return unused strips to the foil pouch containing the desiccant pack. May be stored up to 1 month at 2-8°C.*</td></tr>
<tr><td colspan="3">* Provided this is within the expiration date of the kit</td></tr>
</table>

REFERENCES

1. Lassalle P, Molet S, Janin A, et al (1996) ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines. J Biol Chem 271:20458–20464
2. Tsai JC, Zhang J, Minami T, et al (2002) Cloning and characterization of the human lung endothelial-cell-specific molecule-1 promoter. J Vasc Res 39:148–159. https://doi.org/57763
3. Béchard D, Gentina T, Delehedde M, et al (2001) Endocan is a novel chondroitin sulfate/dermatan sulfate proteoglycan that promotes hepatocyte growth factor/scatter factor mitogenic activity. J Biol Chem 276:48341–48349. https://doi.org/10.1074/jbc.M108395200
4. Sarrazin S, Lyon M, Deakin JA, et al (2010) Characterization and binding activity of the chondroitin/dermatan sulfate chain from Endocan, a soluble endothelial proteoglycan. Glycobiology 20:1380–1388. https://doi.org/10.1093/glycob/cwq100
5. Scherpereel A, Gentina T, Grigoriu B, et al (2003) Overexpression of endocan induces tumor formation. Cancer Res 63:6084–6089
6. Almog N, Ma L, Raychowdhury R, et al (2009) Transcriptional switch of dormant tumors to fast-growing angiogenic phenotype. Cancer Res 69:836–844. https://doi.org/10.1158/0008-5472.CAN-08-2590
7. Jin H, Rugira T, Ko YS, et al (2020) ESM-1 Overexpression is Involved in Increased Tumorigenesis of Radiotherapy-Resistant Breast Cancer Cells. Cancers 12:. https://doi.org/10.3390/cancers12061363
8. Kang YH, Ji NY, Lee CI, et al (2011) ESM-1 silencing decreased cell survival, migration, and invasion and modulated cell cycle progression in hepatocellular carcinoma. Amino Acids 40:1003–1013. https://doi.org/10.1007/s00726-010-0729-6
9. Matano F, Yoshida D, Ishii Y, et al (2014) Endocan, a new invasion and angiogenesis marker of pituitary adenomas. J Neurooncol 117:485–491. https://doi.org/10.1007/s11060-014-1377-6
10. Satchi-Fainaro R, Ferber S, Segal E, et al (2012) Prospective identification of glioblastoma cells generating dormant tumors. PloS One 7:e44395. https://doi.org/10.1371/journal.pone.0044395
11. Béchard D, Scherpereel A, Hammad H, et al (2001) Human endothelial-cell specific molecule-1 binds directly to the integrin CD11a/CD18 (LFA-1) and blocks binding to intercellular adhesion molecule-1. J Immunol Baltim Md 1950 167:3099–3106
12. Zheng X, Soroush F, Long J, et al (2017) Murine glomerular transcriptome links endothelial cell-specific molecule-1 deficiency with susceptibility to diabetic nephropathy. PloS One 12:e0185250. https://doi.org/10.1371/journal.pone.0185250
13. Gaudet A, Portier L, Prin M, et al (2019) Endocan regulates acute lung inflammation through control of leukocyte diapedesis. J Appl Physiol Bethesda Md 1985 127:668–678. https://doi.org/10.1152/japplphysiol.00337.2019
14. Gaudet A, Portier L, Mathieu D, et al (2020) Cleaved endocan acts as a biologic competitor of endocan in the control of ICAM-1-dependent leukocyte diapedesis. J Leukoc Biol 107:833–841. https://doi.org/10.1002/JLB.3AB0320-612RR
15. Bechard D, Meignin V, Scherpereel A, et al (2000) Characterization of the secreted form of endothelial-cell-specific molecule 1 by specific monoclonal antibodies. J Vasc Res 37:417–425. https://doi.org/25758
16. Scherpereel A, Depontieu F, Grigoriu B, et al (2006) Endocan, a new endothelial marker in human sepsis. Crit Care Med 34:532–537
17. Rennel E, Mellberg S, Dimberg A, et al (2007) Endocan is a VEGF-A and PI3K regulated gene with increased expression in human renal cancer. Exp Cell Res 313:1285–1294. https://doi.org/10.1016/j.yexcr.2007.01.021
18. Roudnicky F, Poyet C, Wild P, et al (2013) Endocan is upregulated on tumor vessels in invasive bladder cancer where it mediates VEGF-A-induced angiogenesis. Cancer Res 73:1097–1106. https://doi.org/10.1158/0008-5472.CAN-12-1855
19. Daly C, Pasnikowski E, Burova E, et al (2006) Angiopoietin-2 functions as an autocrine protective factor in stressed endothelial cells. Proc Natl Acad Sci U S A 103:15491–15496. https://doi.org/10.1073/pnas.0607538103
20. Daly C, Wong V, Burova E, et al (2004) Angiopoietin-1 modulates endothelial cell function and gene expression via the transcription factor FKHR (FOXO1). Genes Dev 18:1060–1071. https://doi.org/10.1101/gad.1189704
21. De Freitas Caires N, Gaudet A, Portier L, et al (2018) Endocan, sepsis, pneumonia, and acute respiratory distress syndrome. Crit Care Lond Engl 22:280. https://doi.org/10.1186/s13054-018-2222-7
22. Kao S-J, Chuang C-Y, Tang C-H, et al (2014) Plasma endothelial cell-specific molecule-1 (ESM-1) in management of community-acquired pneumonia. Clin Chem Lab Med 52:445–451. https://doi.org/10.1515/cclm-2013-0638
23. Tang L, Zhao Y, Wang D, et al (2014) Endocan levels in peripheral blood predict outcomes of acute respiratory distress syndrome. Mediators Inflamm 2014:625180. https://doi.org/10.1155/2014/625180
24. Orbegozo D, Rahmania L, Irazabal M, et al (2017) Endocan as an early biomarker of severity in patients with acute respiratory distress syndrome. Ann Intensive Care 7:93. https://doi.org/10.1186/s13613-017-0311-4
25. Ying J, Zhou D, Gu T, Huang J (2019) Endocan, a Risk Factor for Developing Acute Respiratory Distress Syndrome among Severe Pneumonia Patients. Can Respir J 2019:2476845. https://doi.org/10.1155/2019/2476845
26. Perrotti A, Chenevier-Gobeaux C, Ecarnot F, et al (2017) Is Endocan a Diagnostic Marker for Pneumonia After Cardiac Surgery? The ENDOLUNG Study. Ann Thorac Surg. https://doi.org/10.1016/j.athoracsur.2017.07.031

27. Perrotti A, Chenevier-Gobeaux C, Ecarnot F, et al (2017) Relevance of Endothelial Cell-Specific Molecule 1 (Endocan) Plasma Levels for Predicting Pulmonary Infection after Cardiac Surgery in Chronic Kidney Disease Patients: The Endolung Pilot Study. Cardiorenal Med 8:1–8. https://doi.org/10.1159/000479337
28. Bouglé A, Allain P-A, Favard S, et al (2018) Postoperative serum levels of Endocan are associated with the duration of norepinephrine support after coronary artery bypass surgery. Anaesth Crit Care Pain Med. https://doi.org/10.1016/j.accpm.2018.02.013
29. Abd El Halim, Ashraf, Sayed, Manal (2015) Serum endocan role in diagnosis and prognosis of ventilator associated pneumonia. Egypt Soc Chest Dis Tuberc 64:865–869
30. Kupeli I, Salcan S, Kuzucu M, Kuyrukluyıldız U (2018) Can endocan be a new biomarker in ventilator-associated pneumonia? Kaohsiung J Med Sci 34:689–694. https://doi.org/10.1016/j.kjms.2018.07.002
31. Gaudet A, Parmentier E, Dubucquoi S, 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. https://doi.org/10.1016/j.jcrc.2018.06.018
32. Gaudet A, Parmentier E, Dubucquoi S, et al (2019) The complex kinetics of blood endocan during the time course of sepsis and acute respiratory distress syndrome. Crit Care Lond Engl 23:86. https://doi.org/10.1186/s13054-019-2383-z
33. Mikkelsen ME, Shah CV, Scherpereel A, et al (2012) Lower serum endocan levels are associated with the development of acute lung injury after major trauma. J Crit Care 27:522.e11–17. https://doi.org/10.1016/j.jcrc.2011.07.077
34. Tsangaris I, Tsantes A, Vrigkou E, et al (2017) Angiopoietin-2 Levels as Predictors of Outcome in Mechanically Ventilated Patients with Acute Respiratory Distress Syndrome. Dis Markers 2017:6758721. https://doi.org/10.1155/2017/6758721
35. Palud A, Parmentier-Decrucq E, Pastre J, et al (2015) Evaluation of endothelial biomarkers as predictors of organ failures in septic shock patients. Cytokine 73:213–218. https://doi.org/10.1016/j.cyto.2015.02.013
36. Ioakeimidou A, Pagalou E, Kontogiorgi M, et al (2017) Increase of circulating endocan over sepsis follow-up is associated with progression into organ dysfunction. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. https://doi.org/10.1007/s10096-017-2988-6
37. Altintas N, Mutlu LC, Akkoyun DC, et al (2015) Effect of CPAP on New Endothelial Dysfunction Marker, Endocan, in People With Obstructive Sleep Apnea. Angiology. https://doi.org/10.1177/0003319715590558
38. Balta S, Ozturk C (2015) Endocan, Obstructive Sleep Apnea, and Vascular Risk. Angiology. https://doi.org/10.1177/0003319715591332
39. Kanbay A, Ceylan E, Köseoğlu Hİ, et al (2018) Endocan: a novel predictor of endothelial dysfunction in obstructive sleep apnea syndrome. Clin Respir J 12:84–90. https://doi.org/10.1111/crj.12487

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