

# Pregnostic<sup>®</sup>-PE *Ip*



Enzyme-linked Immunosorbent Assay Kit  
for Endothelial Cell-Specific Molecule 1 (ESM-1)



Manual



IQP-303



96 Tests



2-8 °C

For Research Use Only

Not for use in Clinical Diagnostic Procedures

**Pregnostic<sup>®</sup>-PE Ip** (product code IQP-303) contains all the components required for quantification of human Endocan / ESM-1 in cell culture supernatants, serum or plasma.

## **BACKGROUND**

### **What is endocan?**

Endocan, also known as endothelial cell-specific molecule 1 (ESM-1), was originally discovered in endothelial cells <sup>(1)</sup>. The preferred expression in the lung is governed by the proximal promoter region <sup>(2)</sup>. 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 <sup>(3,4)</sup>.

### **What does endocan do?**

Endocan is co-mitogenic through its glycan chain by inducing proliferation of HEK293 cells in the presence of HGF/SF <sup>(3)</sup>. Endocan is protumoral when overexpressed in tumor cells transplanted into SCID mice <sup>(5,6)</sup>. Endocan can also induce migration of endothelial cells when stimulated with VEGF <sup>(7,8)</sup>. Endocan binds the CD11a/CD18 (LFA-1) integrin on human leukocytes, and inhibits its binding to ICAM-1 <sup>(9)</sup>.

### **How endocan is regulated?**

There is a spontaneous synthesis and secretion by endothelial cells. This expression can be increased by the proinflammatory cytokines TNF- $\alpha$  or IL-1 $\beta$  <sup>(10)</sup>, by bacterial lipopolysaccharide <sup>(11)</sup>, or by the angiogenic factors FGF-2 or VEGF <sup>(5,12,13)</sup>. Instead, this expression can be reduced by IFN- $\gamma$  <sup>(10)</sup>, or angiopoietin-1 via the transcription factor FOXO1 <sup>(14,15)</sup>.

Only certain highly specialized endothelial cells appear to overexpress endocan, such as endothelial cells called "tip cells" located at the angiogenic buds in mouse models of angiogenesis <sup>(16,17,18)</sup>, and recently observed at the bladder cancer <sup>(19)</sup>. Endocan overexpression occurs during the angiogenic switch that accompanies the growth of experimental tumors <sup>(20,21)</sup>.

## Clinical studies

Clinical context	n	Endocan Value (ng/mL)	Significance	Ref.
Sepsis	63	0.1 – 30	Sepsis (n=29) : $1.95 \pm 1.63$ ; severe sepsis (n=12) : $1.97 \pm 7.80$ ; septic shock (n=22) : $6.11 \pm 12.99$	11
Polytrauma	48	0.1 – 13	Prediction of respiratory failure occurrence	21
Sepsis	16	0.52 – 6.76	Detection of sepsis-related death	22
Community-acquired pneumoniae	82	0.15 – 11	Correlation with pneumoniae severity scores, Decrease following antibiotic therapy	23
Pulmonary thromboembolism	46	0.32 – 2.51	Associated with severity	24
Hypertension	18		Correlation with carotid intima-media thickness	25
Hypertension	24		Decrease under amlodipin therapy	26
Behçet disease	33	0.58 – 2.99	Se : 76%, Sp : 80%, AUC : 0.835	27
Psoriasis vulgaris	29		Correlation with severity index	28
Cirrhosis	170	> 5	Independently associated with death	29
Renal cell carcinoma	14	0.15 – 16	Increase in clear cell RCC	30
Acute Myeloid leukemia	40	0.80 – 20	Increase associated with bacterial infection, Decrease following antibiotic therapy	31
Lung cancer	50	$3.34 \pm 1.24$	Increase in lung cancer	5
Lung cancer	30	1.21 – 4.30	Bad prognosis at 5-year (cut-off > 1.3)	13

## PRINCIPLE OF THE SANDWICH ELISA

This human ESM-1 ELISA set 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 HUMAN ESM-1 ELISA SET

- 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.

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## 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** - One 96 well microplate (6 strips of 16 wells) pre-coated with the Capture Antibody.

**Human Endocan standard** - 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** - One bottle with 12 mL of Streptavidin-HRP (ready-to-use).

**ELISA Buffer** - One bottle with 75 mL of a 20-fold concentrated solution.

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

**Stop Solution** - One bottle with 12 mL of 2N sulfuric acid.

**Plate sealers** - Two adhesive strips.

## 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

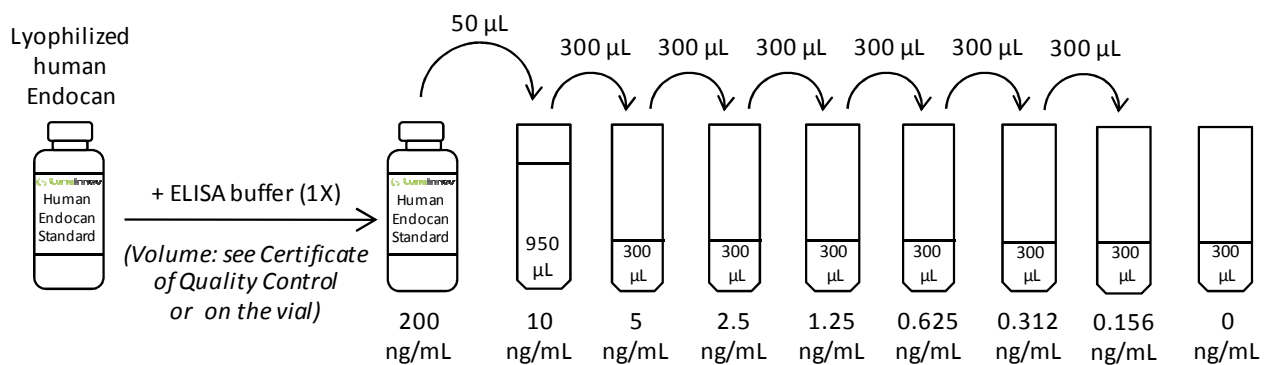
**Plasma** - Collect blood in tube with EDTA as anticoagulant. Centrifuge at 1500 x g for 10 minutes at 4°C. Remove plasma and aliquot before storage at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**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  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Cell Culture Supernatant** - Remove cellular debris by centrifugation and aliquot the supernatant before storage at  $\leq -20^{\circ}\text{C}$ . 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 200 ng/mL.
3. After reconstitution, keep Standard on ice. 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 50  $\mu$ L of the reconstituted Standard solution into 950  $\mu$ L of ELISA Buffer (1X).
5. Add 300  $\mu$ L of ELISA Buffer (1X) to 6 tubes (always use polypropylene tubes).
6. Perform serial dilutions by adding 300  $\mu$ L 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 1:8.
- 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  $\mu$ L 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  $\mu$ L of **ELISA Buffer** (1X).
3. Add 100  $\mu$ L 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  $\mu$ L of **ELISA Buffer** as in step 2.
5. Add 100  $\mu$ L 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  $\mu$ L of **ELISA Buffer** 1X as in step 2.
7. Add 100  $\mu$ L of **TMB** to each well and incubate for 10 min at RT until a blue byproduct is observed. Keep away from light.
8. Add 50  $\mu$ L 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  $\mu$ L of Standard and samples to each well.  
Incubate 1 hour.



Wash 3 times



Add 100  $\mu$ L of Detection Antibody.  
Incubate 1 hour.



Wash 3 times



Add 100  $\mu$ L of Streptavidin-HRP.  
Incubate 30 minutes. Protect from light.



Wash 3 times



Add 100  $\mu$ L of TMB.  
Incubate 10 minutes. Protect from light.



Add 50  $\mu$ L of Stop Solution.



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



### CALCULATION OF RESULTS

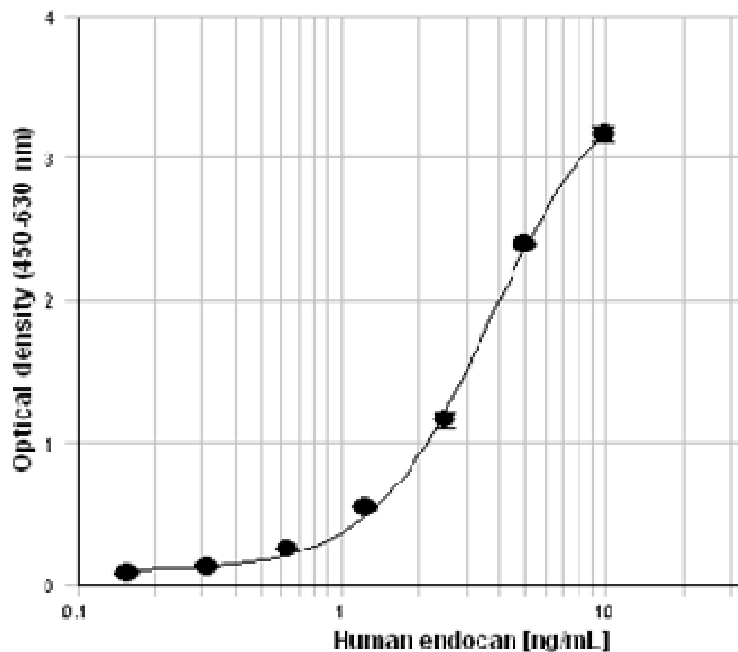
Subtract the zero standard optical density to the optical density of each Standard and each Sample.

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	Lunginnov
Serum	n=32	1.18	0.40 - 2.90	0.46	France	Lunginnov
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

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

\* Provided this is within the expiration date of the kit







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### Explanation of used symbols

	Consult instructions for use
	Catalogue number
	Sufficient for
	Temperature limitation (°C)
	For Research Use Only
	Batch code
	Use by yyyy-mm-dd
	Manufacturer

### MANUFACTURED BY:

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