**Nori® Human Endocan-1 ELISA Kit- DataSheet**

Endothelial cell-specific molecule 1 is a protein that in humans is encoded by the ESM1 gene.[1] This protein is mainly expressed in the endothelial cells in human lung and kidney tissues and is regulated by cytokines, suggesting that it may play a role in endothelium-dependent pathological disorders. The ESM-1 gene product is also called endocan since 2001, when it was characterized as a dermatan sulfate proteoglycan by Bechard et al. Recently, endocan / ESM-1 has been described as a specific biomarker of tip cells during neoangiogenesis by independent teams. Endocan expression has been shown to be increase in presence of pro-angiogenic growth factors such as VEGF or FGF-2. In hypervascularized cancers, overexpression of endocan has been detected by immunohistochemistry using monoclonal antibodies against endocan / ESM-1. Circulating P-selectin levels were closely associated with endocan levels in obese children and adolescents with non-alcoholic fatty liver disease.[2] Inhibition of endocan attenuates monocrotaline-induced connective tissue disease related pulmonary arterial hypertension.[3] Subclinical hypothyroidism is associated with increased levels of serum endocan, dimethylarginine, and TGF-beta, which are new markers for endothelial dysfunction.[4] Endocan reduces the malign grade of gastric cancer cells by regulating associated protein expression.[5] Human endothelial-cell specific molecule-1 binds directly to the integrin CD11a/CD18 (LFA-1) and blocks binding to intercellular adhesion molecule-1.[6]

**References**


**PRINCIPLE OF THE ASSAY**

This ELISA kit is for quantification of endocan-1 in human. This is a quick ELISA assay that reduces time to 50% compared to the conventional method, and the entire assay only takes 3 hours. This assay employs the quantitative sandwich enzyme immunoassay technique and uses biotin-streptavidin chemistry to improve the performance of the assays. An antibody specific for human endocan-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any endocan-1 present is bound by the immobilized antibody. After washing away any unbound substances, a detection antibody specific for human endocan-1 is added to the wells. Following wash to remove any unbound antibody reagent, a detection reagent is added. After intensive wash a substrate solution is added to the wells and color develops in proportion to the amount of endocan-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

This package insert must be read in its entirety before using this product.

**Storage**

Store at 4 °C. The kit should be used in 3 months.
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MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
<th>Description</th>
<th>Quantity</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Precoated Plate</td>
<td>1</td>
<td>20 x PBS</td>
<td>1</td>
<td>Substrate Solution</td>
<td>1</td>
</tr>
<tr>
<td>Detection Antibody</td>
<td>1</td>
<td>20 x Assay Buffer</td>
<td>1</td>
<td>Stop Solution</td>
<td>1</td>
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<tr>
<td>Conjugate</td>
<td>1</td>
<td>Reagent Diluent</td>
<td>1</td>
<td>DataSheet/Manual</td>
<td>1</td>
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<tr>
<td>Standard</td>
<td>3</td>
<td>MSDS/CoA</td>
<td>1</td>
<td>96-well plate sheet</td>
<td>1</td>
</tr>
</tbody>
</table>

Bring all reagents to room temperature before use.

Reagent Preparations

**Human Endocan-1 Detection Antibody** (1 vial) – The lyophilized Detection Antibody should be stored at -20°C in a manual defrost freezer for up to 3 months, if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains sufficient Detection Antibody for a 96-well plate. Add 200 µL of sterile 1 x PBS to a vial and vortex 30 and allow it to sit for 5 min prior to use. Make 1:50 dilution in Reagent Diluent. Take 200 µL of detection antibody to 10.5 mL of Reagent Diluent to make **Working dilution of detection antibody** if the entire 96-well plate is used. If the partial antibody is used store the rest at -20°C until use.

**Human Endocan-1 Standard** (3 vials) – The lyophilized Human Endocan-1 Standard has a total of 3 vials. Each vial contains the standard sufficient for generating a standard curve. The unreconstituted standard can be stored at -20°C for up to 3 months if not used immediately. Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. Add 500 µL of 1 x Assay Buffer to a vial to make the high standard concentration of 1,600 pg/mL and vortex 30 sec and allow it to sit for 5 min. A seven-point standard curve is generated using 2-fold serial dilutions in 1 x Assay Buffer, vortex 30 sec for each of dilution step.

**Conjugate** (53 µL) – Centrifuge for 1 min at 6000 x g to bring down the material prior to open the vial. The vial contains 53 µL Conjugate sufficient for one 96-well plate. If the volume is less than 53 µL, add sterile 1 x PBS to reach 53 µL and vortex 10 sec. Make 1:200 dilutions in Reagent Diluent. If the entire 96-well plate is used, add 53 µL of Conjugate to 10.5 mL of Reagent Diluent to make **working dilution of Conjugate** prior to the assay. The rest of undiluted Conjugate can be stored at 4°C for up to 3 months. DO NOT FREEZE.

**20 x PBS**, pH 7.3, 30 mL- Dilute to 1 x PBS with deionized distilled water and mix well prior to use.
**20 x Assay Buffer**, 20 mL- Dilute to 1 x Assay Buffer with 1 x PBS prior to use.
**Reagent Diluent**, 21 mL.
**Substrate Solution**, 10.5 mL.
**Stop Solution**, 5.5 mL.
Assay Procedure
1. Lift the plate cover and cover the wells that are not used using the strip provided. Vortex briefly the samples prior to the assay. Add 100 µL of sample (such as plasma or serum) or standard per well and use duplicate wells for each standard or sample. Cover the 96-well plate and incubate 1 hour at room temperature.
2. Aspirate each well and wash with 1 x Assay Buffer, repeating the process two times for a total of three washes. Wash by filling each well with 1 x Assay Buffer (300 µL) using a multi-channel pipette, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Assay Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 µL of the working dilution of Detection Antibody to each well. Cover the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 µL of the working dilution of Conjugate to each well. Cover the plate and incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 µL of Substrate Solution to each well. Incubate for 10-20 minutes at room temperature. Avoid placing the plate in direct light.
8. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Precaution and Technical Notes
1. It is critical to follow the procedure step by step otherwise appropriate color development may not occur as expected.
2. A standard curve should be generated for each set of samples assayed. Thorough mixing of the standards at each step of the dilutions is critical to ensure a normal standard curve.
3. Plasma or serum sample should be diluted with equal volume of 1 x Assay Buffer and vortex for 1 min prior to assay. If the OD value still exceeds the upper limit of the standard curve, further dilution is recommended till it falls in the detection range and the dilution factor must be used for calculation of the concentration.
4. Conjugate contains enzyme, DO NOT mass up with Detection Antibody.
5. The Stop Solution is an acid solution, handle with caution.
6. This kit should not be used beyond the expiration date on the label.
7. A thorough and consistent wash technique is essential for proper assay performance.
8. Use a fresh reagent reservoir and pipette tips for each step.
9. It is recommended that all standards and samples be assayed in duplicate.
10. Avoid microbial contamination of reagents and buffers. This may interfere with the sensitivity of the assay.
Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average zero (blank) standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the endocan-1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

The Standard Curve

The graph below represents typical data generated when using this human endocan-1 ELISA Kit. The standard curve was calculated using a computer generated 4-PL curve-fit. For this case, a Bio-Rad iMark™ Microplate Reader and a Microplate Manager 6 Software were used to generate this curve. The correlation coefficient ($r^2$) is 0.999-1.000.
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Specificity
The following recombinant human proteins prepared at 1 ng/ml were tested and exhibited no cross-reactivity or interference. Adiponectin, ApoAl, BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, CCL2, CCL4, CCL5, CRP, FGF acidic, HGF, HSP27, IGF-1, IL-1α, IL-1β, IL-1ra, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17C, ENDOCAN-11, ENDOCAN-13, IFN-α, IFN-β, IFNγ, MMP-2, MMP-3, MMP-9, PDGF, PLA2G7, prolactin, TLR1, TLR2, TLR3, TLR4, TLR9, TGF-β1, TGF-β2, TGF-β3, TNF-α, TNF RI, TNF RII, VEGF, VEGF-R1.

Calibration
This kit is calibrated against a highly-purified yeast-expressed recombinant human endocan-1.

Detection Range
25-1600 pg/ml

Assay Sensitivity
5 pg/ml

Assay Precision
Intra-Assay %CV: 5; Inter-Assay %CV: 10

For Research Use Only

Related products
20 x sample diluent, GR103061
10 x ELISA Wash Buffer, GR103014
10 x Reagent Diluent, GR103028
20 x PBS, GR103004
ELISA Substrate, GR103021
ELISA Stop Solution, GR103055
ELISA Conjugate, GR103044
Human endocan-1 standard
Human endocan-1 detection antibody
## Troubleshooting Guide

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible causes</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>• Inaccurate pipetting</td>
<td>• Check pipettes</td>
</tr>
<tr>
<td></td>
<td>• Improper standard curve</td>
<td>• Check and use the correct dilution buffer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Vortex 30 sec for each of standard dilution steps</td>
</tr>
<tr>
<td>Low signal</td>
<td>• Improper preparation of standard, samples, detection antibody, and/or conjugate</td>
<td>• Briefly spin down vials before opening. Reconstitute the powder thoroughly.</td>
</tr>
<tr>
<td></td>
<td>• Too brief incubation times</td>
<td>• Ensure sufficient incubation time.</td>
</tr>
<tr>
<td></td>
<td>• Inadequate reagent volume or improper dilution</td>
<td>• Check pipettes and ensure correct preparation.</td>
</tr>
<tr>
<td>Large CV</td>
<td>• Inaccurate pipetting and mixing</td>
<td>• Check pipettes and ensure thorough mixing.</td>
</tr>
<tr>
<td></td>
<td>• Improper standard/sample dilutions.</td>
<td>• Use the correct dilution buffers</td>
</tr>
<tr>
<td></td>
<td>• Air bubbles in wells</td>
<td>• Remove bubbles in wells.</td>
</tr>
<tr>
<td>High background</td>
<td>• Plate is insufficiently washed.</td>
<td>• Review the datasheet for proper wash. If using a plate washer, ensure that all ports are unobstructed.</td>
</tr>
<tr>
<td></td>
<td>• Contaminated Assay Buffer</td>
<td>• Make fresh Assay Buffer</td>
</tr>
<tr>
<td>No signal detected</td>
<td>• The procedure was misconduct.</td>
<td>• Ensure the step-by-step protocol was correctly followed and no misstep was conducted.</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>• Improper storage of the ELISA kit</td>
<td>• Store standards and detection antibody at -20°C after reconstitution, others at 4°C. Keep substrate protected from light.</td>
</tr>
<tr>
<td></td>
<td>• Stop solution</td>
<td>• Adding stop solution to each well before reading plate</td>
</tr>
</tbody>
</table>