

Code No. 27188

**Human Syndecan-4 Assay Kit - IBL****INTRODUCTION**

The syndecan family contains four members (syndecan-1 / syndecan, syndecan-2 / fibroglycan, syndecan-3 / N-syndecan, syndecan-4 / ryudocan (amphyglycan)), which are transmembrane heparan sulfate proteoglycans (HSPGs). These HSPGs exhibit cell type-specific distribution with vascular endothelial cells expressing syndecan-1, -2, and -4, and predominant targeting to basolateral surfaces. The syndecan family members are type I integral membrane proteins with homologous transmembrane and cytoplasmic domains. The combined transmembrane / cytoplasmic domains contain four well-conserved tyrosine residues, which might serve important roles for biological function. The cytoplasmic tail of syndecan-4 interacts with focal adhesion molecules.

Thus, syndecan-4 is known to have various biological functions such as anticoagulation, cell adhesion and cell multiplication.

**PRINCIPLE**

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Human Syndecan-4.

**MEASUREMENT RANGE**

20 ~ 1,280 pg/mL

**INTENDED USE**

- This IBL's assay kit is capable for the quantitative determination Human Syndecan-4 in serum, EDTA-plasma and cell culture supernatant.
- It is not recommend to use human heparin-plasma as a sample in this kit.

**KIT COMPONENT**

|   |  |            |
|---|--|------------|
| 1 | Precoated plate :Anti-Human Syndecan-4 Rabbit IgG Affinity Purify            | 96Well x 1 |
| 2 | Labeled antibody Conc.   |            |
|   | : (30X) HRP conjugated Anti- Human Syndecan-4Rabbit IgG Fab' Affinity Purify | 0.4mL x 1  |
| 3 | Standard : Human Syndecan-4  | 0.5mL x 2  |
| 4 | EIA buffer*  | 30mL x 1   |
| 5 | Solution for Labeled antibody*   | 12mL x 1   |
| 6 | Chromogen : TMB solution   | 15mL x 1   |
| 7 | Stop solution*   | 12mL x 1   |
| 8 | Wash buffer Conc.*   | 50mL x 1   |

**OPERATION MANUAL****1. Materials needed but not supplied**

- Plate reader (450nm)
- Graduated cylinder and beaker
- Refrigerator (as 4°C)
- Paper towel
- Incubator (37°C ± 1°C)
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
- Micropipette and tip
- Deionized water
- Graph paper (log/log)
- Tube for dilution of Standard

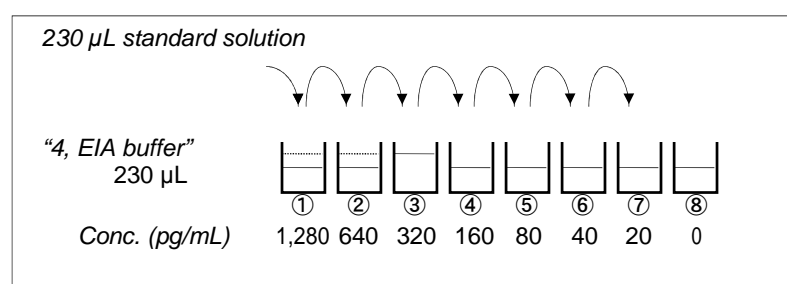
**2. Preparation**

- 1) Preparation of wash buffer  
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
- 2) Preparation of Labeled antibody  
"2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.  
Example)  
In case you use one strip (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of "2, Labeled antibody Conc." with 870 µL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 µL in each well.)  
This operation should be done just before the application of Labeled antibody.  
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
- 3) Preparation of Standard  
Put just 0.5 mL of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 2,560 pg/mL Human Syndecan-4 standard.
- 4) Dilution of Standard  
Prepare 8 tubes for dilution of "3, Standard". Put 230 µL each of "4, EIA buffer" into the tube.  
Specify the following concentration of each tube."  

|        |                             |
|--------|-----------------------------|
| Tube-1 | 1,280 pg/mL                 |
| Tube-2 | 640 pg/mL                   |
| Tube-3 | 320 pg/mL                   |
| Tube-4 | 160 pg/mL                   |
| Tube-5 | 80 pg/mL                    |
| Tube-6 | 40 pg/mL                    |
| Tube-7 | 20 pg/mL                    |
| Tube-8 | 0 pg/mL (Test Sample Blank) |

Put 230 µL of Standard solution into tube-1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 1,280 pg/mL and 20 pg/mL. Tube-8 is the test sample blank as 0 pg/mL.

See following picture.

**5) Dilution of test sample**

Test sample may be diluted with "4, EIA buffer" or PBS as necessary.

ex) Serum or EDTA-plasma samples are recommended to be diluted more than 30-fold.

If the concentration of Human Syndecan-4 in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

**3. Measurement procedure**

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

|  | Test Sample        | Standard                           | Test Sample Blank          | Reagent Blank     |
|--|--------------------|------------------------------------|----------------------------|-------------------|
| Reagents   | Test sample 100 µL | Diluted standard (Tube 1~7) 100 µL | EIA buffer (Tube-8) 100 µL | EIA buffer 100 µL |
| Incubation for 60 minutes at 37°C with plate lid   |                    |                                    |                            |                   |
| 4 times (wash buffer more than 350 µL)*  |                    |                                    |                            |                   |
| Labeled Antibody   | 100 µL             | 100 µL                             | 100 µL                     | -                 |
| Incubation for 30 minutes at 4°C with plate lid  |                    |                                    |                            |                   |
| 5 times (wash buffer more than 350 µL)*  |                    |                                    |                            |                   |
| Chromogen  | 100 µL             | 100 µL                             | 100 µL                     | 100 µL            |
| Incubation for 30 minutes at room temperature (shielded)   |                    |                                    |                            |                   |
| Stop solution  | 100 µL             | 100 µL                             | 100 µL                     | 100 µL            |
| Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution. |                    |                                    |                            |                   |

- 1) Determine wells for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
- 2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
- 3) Incubate the precoated plate for 60 minutes at 37°C after covering it with plate lid.
- 4) Wash the plate with the prepared wash buffer and remove all liquid.\*
- 5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
- 6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
- 7) Wash the plate with the prepared wash buffer and remove all liquid.\*
- 8) Take the required quantity of "6, Chromogen" into a disposable test tube. Then, pipette 100µL from the test tube into the wells. Please do not return the rest of the test tube to "6, Chromogen" bottle to avoid contamination.
- 9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by addition of "6, Chromogen".
- 10) Pipette 100 µL of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by addition of "7, Stop solution".
- 11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank. The measurement shall be done within 30 minutes after addition of "7, Stop solution".

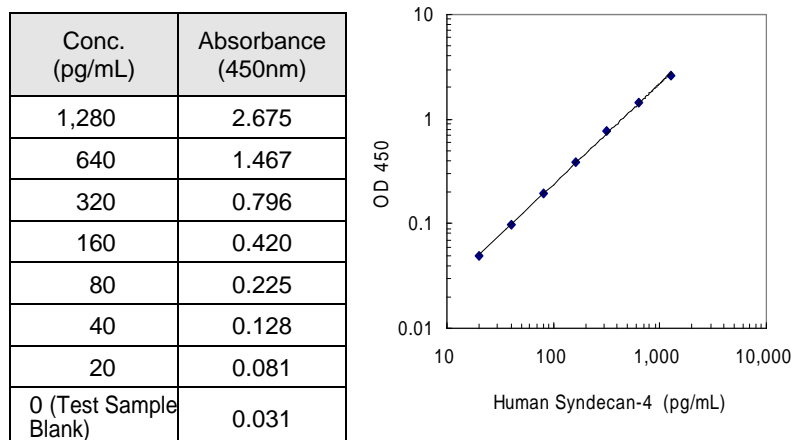
**SPECIAL ATTENTION**

- 1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
- 2) Test samples should be diluted with "4, EIA buffer" or PBS, if the need arises.
- 3) Duplicate measurement of test samples and standard is recommended.
- 4) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
- 5) Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
- 6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
- 7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.
- 8) Measurement should be done within 30 minutes after addition of "7, Stop solution".

## CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

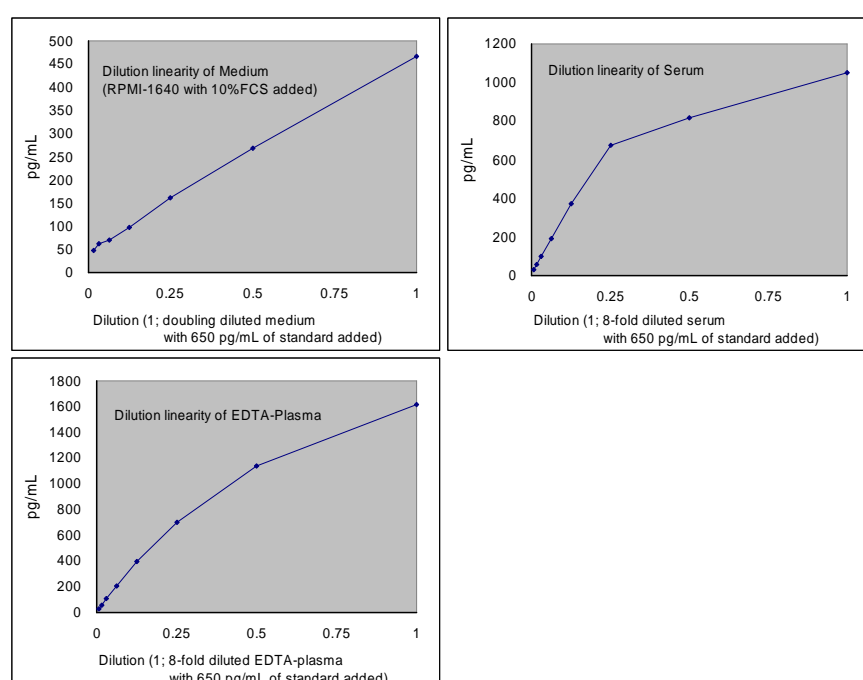
### Example of standard curve



\* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

## PERFORMANCE CHARACTERISTICS

### 1. Dilution Linearity



### 2. Added Recovery Assay

| Specimen                    | Theoretical Value (pg/mL) | Measurement Value (pg/mL) | %    |
|-----------------------------|---------------------------|---------------------------|------|
| 10%FCS added RPMI-1640 (x4) | 1304.48                   | 1081.25                   | 82.9 |
|                             | 654.48                    | 548.19                    | 83.8 |
|                             | 329.48                    | 276.65                    | 84.0 |
|                             | 166.98                    | 140.70                    | 84.3 |
| Human Serum (x16)           | 1030.15                   | 1017.60                   | 98.8 |
|                             | 867.65                    | 722.38                    | 83.3 |
|                             | 786.40                    | 685.54                    | 87.2 |
|                             | 745.78                    | 675.03                    | 90.5 |
| Human Plasma (EDTA) (x16)   | 1257.57                   | 1153.32                   | 91.7 |
|                             | 1095.07                   | 1087.05                   | 99.3 |
|                             | 1013.82                   | 994.89                    | 98.1 |
|                             | 973.20                    | 916.55                    | 94.2 |

### 3. Intra – Assay

| Measurement Value (pg/mL) | SD value | CV value (%) | n  |
|---------------------------|----------|--------------|----|
| 721.48                    | 35.10    | 4.9          | 21 |
| 240.68                    | 8.20     | 3.4          | 21 |
| 79.49                     | 3.24     | 4.1          | 21 |

### 4. Inter - Assay

| Measurement Value (pg/mL) | SD value | CV value (%) | n |
|---------------------------|----------|--------------|---|
| 718.06                    | 4.02     | 0.6          | 3 |
| 245.52                    | 4.85     | 2.0          | 3 |
| 80.69                     | 1.63     | 2.0          | 3 |

### 5. Specificity

| Compound          | Cross Reactivity |
|-------------------|------------------|
| Human Syndecan-4  | 100 %            |
| Human Tenascin-C  | <0.1%            |
| Human Osteopontin | <0.1%            |
| Human IgG         | <0.1%            |

### 6. Sensitivity

3.94 pg/mL

The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

## PRECAUTION FOR INTENDED USE AND/OR HANDLING

- All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
- "3, Standard" is lyophilized products. Be careful to open this vial.
- "7, Stop solution" is a strong acid substance. Therefore, be careful not to have your skin and clothes contact "7, Stop solution" and pay attention to the disposal of "7, Stop solution".
- Dispose used materials after rinsing them with large quantity of water.
- Precipitation may occur in "2, Labeled antibody Conc.", however, there is no problem in the performance.
- Wash hands after handling reagents.
- Do not mix the reagents with the reagents from a different lot or kit.
- Do not use expired reagents.
- This kit is for research purpose only. Do not use for clinical diagnosis.

## STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C

The expiry date is specified on outer box.

## REFERENCE

- Kojima T, Takagi A, Maeda M, Segawa T, Shimizu A, Yamamoto K, Matsushita T, Saito H. Plasma levels of syndecan-4 (ryudocan) are elevated in patients with acute myocardial infarction. *Thromb Haemost.* 2001 May;85(5):793-9.

Version 2.

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Made in Japan.