Human Syndecan-1 ELISA KIT

Catalog Number	Size
CHE0300	96 Tests

Human Syndecan-1 ELISA KIT

For the quantitative determination of Human Syndecan-1 concentrations in cell culture supernates, serum, and plasma.This package insert must be read in its entirety before using this product. If you have questions or experience problems with this product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
INTRODUCTION

Syndecan-1, designated CD138, is a dimeric type I transmembrane (TM) protein that belongs to the syndecan family of Type 1 transmembrane proteins . The four syndecan family members are major carriers of heparan sulfate (HS) and chondroitin sulfate glycosaminoglycans (GAGs) that have different expression patterns and extracellular sequences. Syndecan-1 forms weak non-covalent homodimers, or heterodimers with Syndecan-2 or -3, through interactions of the transmembrane domain. It is synthesized as a 310 amino acid (aa) precursor with a 17 aa signal sequence, a 234 aa extracellular domain (ECD) that includes three closely-spaced consensus Ser-Gly HS attachment sites near the N-terminus, a 25 aa TM segment, and a 34 aa cytoplasmic region that includes a PDZ binding motif with a tyrosine phosphorylation site. The ECD is variably modified by GAGs, producing molecular weights of 120 - 200 kDa for native Syndecan-1. Soluble forms are shed via proteolytic cleavage. Human Syndecan-1 ECD shares 65 - 71% aa identity with the ECD of rat, mouse, canine, equine and bovine Syndecan-1. Syndecan-1 shows highest expression on epithelial cells such as keratinocytes, and terminally differentiated B cells such as plasma cells. It aids wound healing in skin, cornea, and heart following myocardial infarction by promoting re-epithelialization, migration, and collagen deposition. It binds chemokines, creating chemotactic gradients when shed, but also binds and modulates integrins to control the influx of leukocytes. The net effect is to allow, but limit, inflammation. In myeloma and other cancers, shedding of Syndecan-1 can facilitate growth, angiogenesis and metastasis. Growth factors, such as FGFs and HGF, bind GAG chains and use Syndecan-1 as a coreceptor. The GAG chains may also be used by a variety of viruses and bacteria for cell adhesion and uptake.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Syndecan-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Syndecan-1 present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for Syndecan-1 is added to the wells and binds to the combination of capture antibody-Syndecan-1 in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the

amount of Syndecan-1 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven Syndecan-1 standard dilutions and Syndecan-1 sample concentration determined.

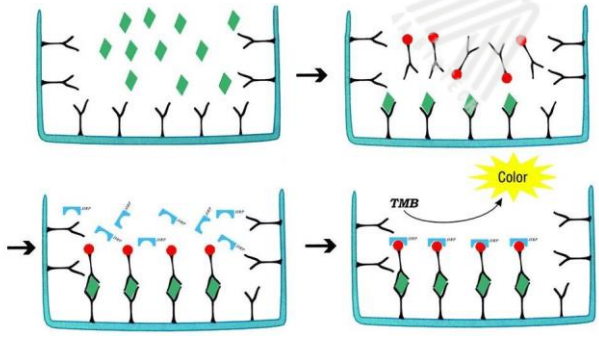

Figure 1:Schematic diagram of the assay

REAGENTS

1. Aluminium pouches with a Microwell Plate coated with antibody to Human Syndecan-1 (8 x 12)
2. 2 vials Human Syndecan-1 Standard lyophilized, 8000 pg/ml upon reconstitution
3. 2 vials concentrated Biotin-Conjugate anti-Human Syndecan-1 antibody
4. 2 vials Streptavidin-HRP solution
5. 1 bottle Standard /sample Diluent
6. 1 bottle Biotin-Conjugate antibody Diluent
7. 1 bottle Streptavidin-HRP Diluent
8. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)
9. 1 vial Substrate Solution
10. 1 vial Stop Solution
11. 4 pieces Adhesive Films
12. package insert

NOTE: [96 Tests]

STORAGE

Table 1: Storage of the kit

Unopened Kit	Store at 2 – 8℃. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Standard /sample Diluent	May be stored for up to 1 month at 2 – 8℃.**
	Concentrated Biotin-Conjugate	
	Streptavidin-HRP solution	
	Biotin-Conjugate antibody Diluent	
	Streptavidin-HRP Diluent	
	Wash Buffer Concentrate 20x	
	Substrate Solution	
	Standard	Aliquot and store for up to 1 month at -20℃. Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 – 8℃.**

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

THE REQUIRED ITEMS (not provided, but can help to buy):

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000 μL.
3. 37 ℃ incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

PRECAUTIONS FOR USE

1. Store kit regents between 2℃and 8℃. After use all reagents should be immediately returned to cold storage(2℃ to 8℃).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.

4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test

to determine the dilution factor).

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard** - Reconstitute the Standard with 1.0mL of Standard /sample Diluent. This reconstitution produces a stock solution of 8000 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   Pipette 500 μ L of Standard/sample Diluent into the 4000 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 8000 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).

   If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.
4. Working solution of Biotin-Conjugate anti-Human Syndecan-1 antibody: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.
5. Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   The working solution should be used within one day after dilution.

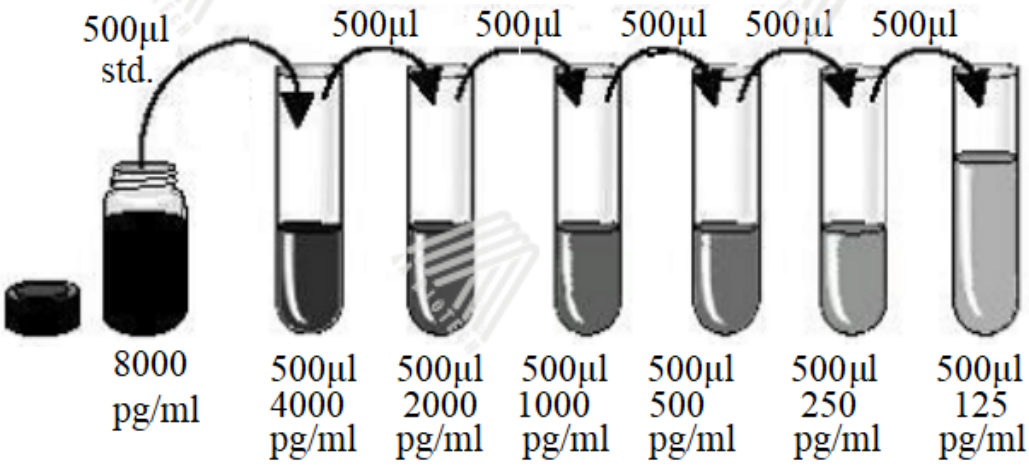

Figure 2: Preparation of Syndecan-1 standard dilutions

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.
3. Add 100 µL of Standard, control, or sample, per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at 37℃.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350 µL) using a squirt bottle, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of the working solution of Biotin-Conjugate to each well. Cover with a new adhesive strip and incubate 1 hours at 37℃.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at 37℃. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.

9. Add 100 μL of Substrate Solution to each well. Incubate for 10-20 minutes at 37℃. Avoid placing the plate in direct light.
10. Add 100 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.(optionally 630nm as the reference wave length;610-650nm is acceptable)

ASSAY PROCEDURE SUMMARY

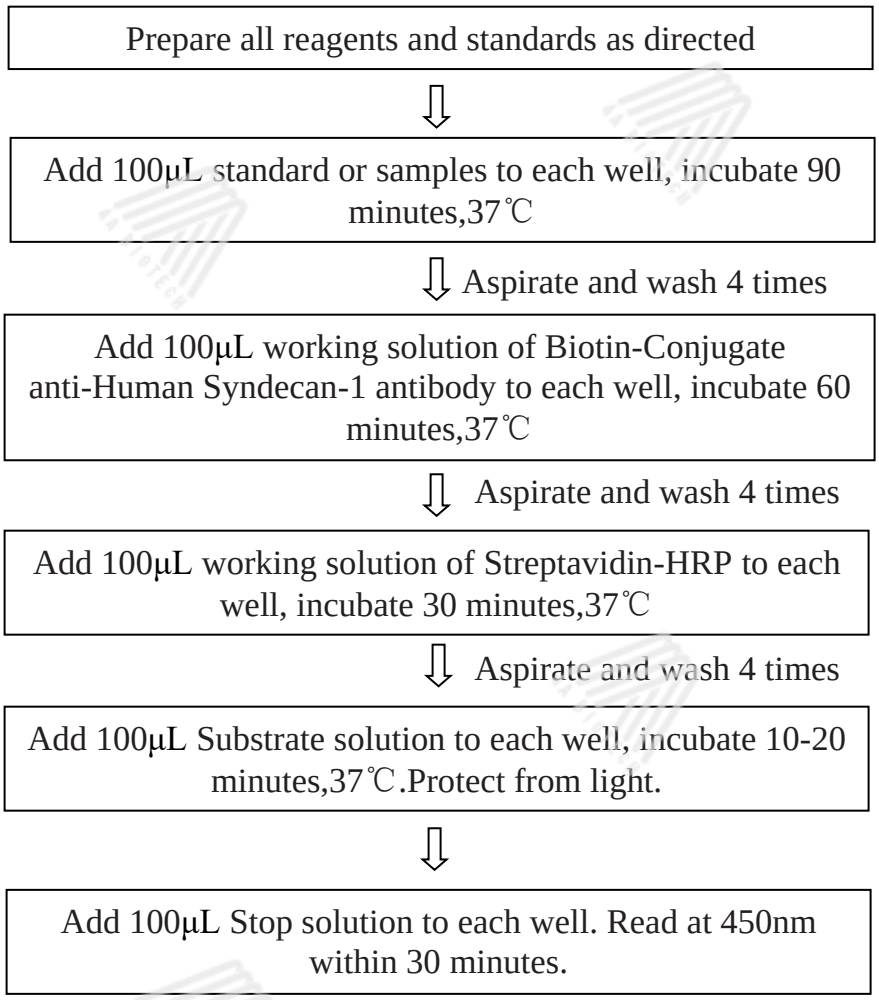

Figure 3: Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. 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.
3. The data may be linearized by plotting the log of the Syndecan-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.
4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 2:Typical data using the Syndecan-1 ELISA (Measuring

wavelength:450nm, Reference wavelength:630nm)

Standared (pg/ml)	OD.	OD.	Average	Corrected
0	0.037	0.036	0.037	——
125	0.083	0.079	0.081	0.044
250	0.142	0.139	0.141	0.104
500	0.228	0.235	0.232	0.195
1000	0.469	0.474	0.472	0.435
2000	0.886	0.893	0.890	0.853
4000	1.443	1.435	1.439	1.402
8000	2.460	2.453	2.457	2.420

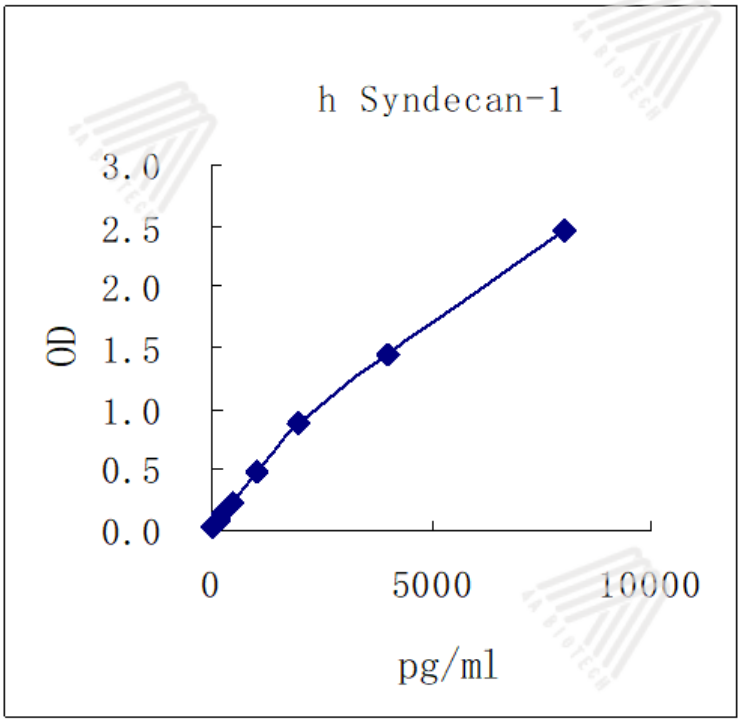

Figure 4:Representative standard curve for Syndecan-1 ELISA. Syndecan-1 was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

SENSITIVITY, SPECIFICITY AND REPEATABILITY

1. **REPEATABILITY**: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
2. **SENSITIVITY**: The minimum detectable dose was 62pg/mL.
3. **SPECIFICITY:** This assay recognizes both natural and recombinant Human Syndecan-1. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 3: Factors assayed for cross-reactivity

Recombinant human	**Recombinant mouse**
ADAMTS4	Syndecan-1
CD44/Fc Chimera	
COL23A2	
Eotaxin	
GRO α	
HGF	
MCP-3	
Midkin	
MMP-7	
RANTES	
Syndecan-2	
Syndecan-3	
Syndecan-4	
TARC	

REFERENCES

1. Circulating syndecan-1 is associated with chemotherapy-resistance in castration-resistant prostate cancer. Szarvas T, et al. Urol Oncol, 2018 Jun.
2. Syndecan-1 suppresses epithelial-mesenchymal transition and migration in human oral

cancer cells. Wang X, et al. Oncol Rep, 2018 Apr.
3. Molecular targets and signaling pathways regulated by nuclear translocation of syndecan-1. Szatmári T, et al. BMC Cell Biol, 2017 Dec 8.
4. Circulating syndecan-1 predicts the development of disseminated intravascular coagulation in patients with sepsis. Ikeda M, et al. J Crit Care, 2018 Feb.
5. Syndecan-1: A Quantitative Marker for the Endotheliopathy of Trauma. Gonzalez Rodriguez E, et al. J Am Coll Surg, 2017 Sep.

RELATED PRODUCTS

Table 4: Related products

Products name	Catalog number	size
Human IL-1 α ELISA Kit	CHE0101	48T/96T
Human IL-1 β ELISA Kit	CHE0001	48T/96T
Human IL-2 ELISA Kit	CHE0003	48T/96T
Human IL-3 ELISA Kit	CHE0028	48T/96T
Human IL-4 ELISA Kit	CHE0005	48T/96T
Human IL-6 ELISA Kit	CHE0009	48T/96T
Human IL-8 ELISA Kit	CHE0011	48T/96T
Human IL-10 ELISA Kit	CHE0013	48T/96T
Human IL-12p40 ELISA Kit	CHE0016	48T/96T
Human IL-12p70 ELISA Kit	CHE0015	48T/96T
Human IFN-γ ELISA Kit	CHE0017	48T/96T
Human TNF-α ELISA Kit	CHE0019	48T/96T
Human APO-1/FAS ELISA Kit	CHE0024	48T/96T
Human TGF-β 1 ELISA Kit	CHE0029	48T/96T
Human MCP-1 ELISA Kit	CHE0103	48T/96T
Human EGF ELISA Kit	CHE0044	48T/96T
Human G-CSF ELISA Kit	CHE0025	48T/96T
Human GM-CSF ELISA Kit	CHE0026	48T/96T
Human sICAM-1 ELISA Kit	CHE0052	48T/96T
Human Leptin ELISA Kit	CHE0053	48T/96T
Human VEGF ELISA Kit	CHE0043	48T/96T

If you have any questions, please tell us!