

Human syndecan-1 ELISA Kit

Sandwich ELISA

Catalog EL-213-1901

INTRODUCTION

Syndecan-1 is a type I transmembrane heparan sulfate (and some chondroitin sulfate) proteoglycan that physically bridges the actin cytoskeleton to extracellular matrix proteins, giving cells a structural handle for adhesion, spreading, and migration. Beyond this scaffolding role, it helps direct exosome formation together with syndecan-binding partners and participates in receptor-mediated uptake of extracellular ligands, including facilitating entry of the HIV-1 Tat protein into cells. Its expression is altered across a range of solid tumors and hematologic malignancies, where changes in syndecan-1 levels have been associated with shifts in tumor cell invasiveness and metastatic behavior, and separately it has drawn interest as a marker of endothelial and vascular integrity in critical illness and tissue injury. A shed, soluble ectodomain of the protein circulates and can be measured in blood or other fluids as an indicator of these underlying cellular processes.

PRINCIPLE OF THE ASSAY

This kit employs a sandwich enzyme immunoassay format in which the microtiter plate wells are pre-coated with a capture antibody specific to human syndecan-1 (SDC1). During the assay, standards or samples are added to the appropriate wells together with a biotin-conjugated detection antibody that also recognizes human SDC1. Avidin conjugated to horseradish peroxidase (HRP) is then added to each well and allowed to incubate, completing the detection complex. Upon addition of TMB substrate solution, a colorimetric signal develops exclusively in wells containing human SDC1, the biotin-conjugated antibody, and avidin-HRP. The enzymatic reaction is stopped by the addition of a sulphuric acid stop solution, and absorbance is measured spectrophotometrically at 450 nm ($\pm$ 10 nm). Sample concentrations are determined by comparing the optical density of each sample against a standard curve generated from the kit-provided standards.

MATERIALS AND STORAGE

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

Each kit includes:	Units
Pre-Coated Microplate	12 strips x 8 wells
Standard (Lyophilized)	2 vials
Biotinylated Antibody (100×)	120 µL
Streptavidin-HRP (100×)	120 µL
Standard/Sample Diluent Buffer	20 mL
Biotinylated Antibody Diluent	12 mL
HRP Diluent	12 mL
Wash Buffer (25×)	20 mL
TMB Substrate Solution	10 mL
Stop Reagent	6 mL
Plate Covers	2 pieces
<i>Do not mix or substitute reagents with those from other lots.</i>	

MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

SAFETY PRECAUTIONS

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective

clothing when handling any patient sera or serum based products.

- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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PREPARATION OF REAGENTS

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

- 1. Wash Buffer (1X) Preparation: Dilute the 25X wash buffer concentrate 1:25 with double-distilled Water.**
- 2. Detection Reagent (1X) Preparation: Dilute the biotinylated antibody and streptavidin-HRP concentrates 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively, immediately before use.**
- 3. Preparation of Calibrators: Prepare calibrators by serial dilution from a 100 down to 1.57 (plus a 0 blank), per the source manual.**

Sol'n ID	Source	Source Vol (μL)	Cal. Diluent (μL)	Final Vol (μL)	Final Conc. (ng/mL)
Std 1	Stock Standard	500	500	1000	100
Std 2	Std 1	500	500	1000	50
Std 3	Std 2	500	500	1000	25
Std 4	Std 3	500	500	1000	12.5
Std 5	Std 4	500	500	1000	6.25
Std 6	Std 5	500	500	1000	3.13
Std 7	Std 6	500	500	1000	1.57
Blank	Std 7	500	500	1000	0

SPECIMEN STORAGE

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

ASSAY PROCEDURE

1. After the kit is equilibrated at room temperature, add 100 μL of Standard Working Buffer (gradually diluted according to the instructions) or 100 μL of sample to each well, and incubate at 37°C for 80 minutes.
2. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 μL Biotinylated Antibody Working Solution (1×) to each well, incubate at 37°C for 50 minutes.
3. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 3 times. After pat it dry against clean absorbent paper, add 100 μL 1× Streptavidin-HRP Working Solution to each well, incubate at 37°C for 50 minutes.
4. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 μL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark.
5. Add 50 μL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

CALCULATIONS AND RESULTS

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
2. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.

3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

PERFORMANCE CHARACTERISTICS

Precision: Intra-assay CV < 8%. Inter-assay CV < 10%.

Detection Limit: 0.69 ng/mL

Recovery: Serum (n = 5): 80-94% (avg 87%); EDTA plasma (n = 5): 95-107% (avg 101%); Heparin plasma (n = 5): 82-95% (avg 88%)

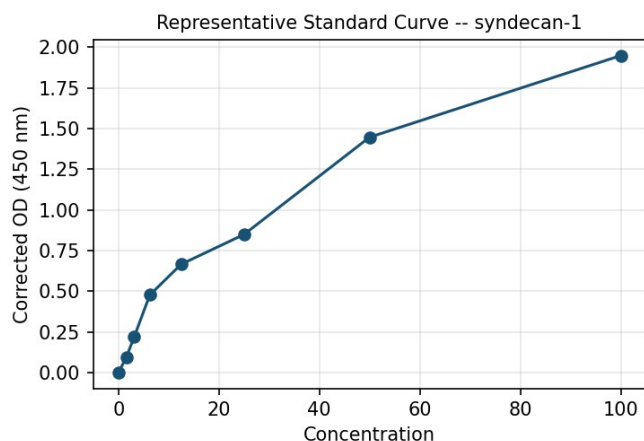
Specificity: This assay has high sensitivity and excellent specificity for detection of Human SDC1. No significant cross-reactivity or interference between Human SDC1 and analogues was observed.



This kit uses a sandwich enzyme immunoassay format to quantify human syndecan-1 in biological samples.

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SAMPLE STANDARD CURVE



Representative standard curve shown for illustration only. This curve should not be used to calculate unknown sample concentrations -- run a new standard curve with each assay.

ORDERING INFORMATION

Please visit www.affinityimmuno.com to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.