

## Mouse sCD138 (Syndecan-1) ELISA KIT

**Catalog No.:** CDK064A    **Lot No.:** TBD    **Size:** 1 Plate (1 x 96 tests)    **Expiration date:** TBD

**Catalog No.:** CDK064B    **Lot No.:** TBD    **Size:** 2 Plates (2 x 96 tests)    **Expiration date:** TBD

**NOTE:** this is a sample protocol which is subject to variation by Lot Number. Refer to the protocol inserted in your package for the current lot number specifications and expiration date or contact our technical support at [tech@cellsciences.com](mailto:tech@cellsciences.com)

<b>Specificity:</b>	Recognizes both native and recombinant mouse soluble Syndecan-1
<b>Sensitivity:</b>	<0.35 ng / mL
<b>Range:</b>	0.5 ng / mL - 16 ng / mL
<b>Sample Type:</b>	Buffered solutions, cell culture supernatant, serum, plasma, or other body fluids
<b>Cross-Reactivity:</b>	No cross reactivity with other mouse molecules tested and <3.7% to human CD138.

### 1. INTRODUCTION

Syndecans are a transmembrane protein family within the heparin sulfate proteoglycan group that interact with many different molecules of the immune system through their heparin sulfate chains. The mammalian syndecan family consists of 4 proteins: Syndecans 1 to 4 are each encoded by distinct genes. In adult tissues, syndecan 1 (CD138) is predominantly expressed by epithelial and plasma cells (both normal and malignant) and is currently considered to be the most reliable surface marker for plasma cells. In addition, CD138 is also expressed on pre and immature B cells; however, this is regulated by IL-6 and LPS stimulation. CD138 has previously been shown to participate in cell-to-cell interactions, organ development, vessel formation and tissue regeneration following injury. CD138 is regularly cleaved from the membrane and, as a consequence, high levels of soluble CD138 are found in the blood, which can be easily detected using a CD138 specific ELISA. CD138 binds to and modulates the activity of a wide range of molecules involved in inflammation via its heparin sulfate chains, including chemokines, growth factors, selectins and other adhesion molecules. CD138 can also act as a receptor for collagen, fibronectin, thrombospondin, and tenascin, and is, therefore, involved in cell matrix adhesion. CD138 mediates the binding of myeloma cells to type I collagen and inhibits tumor cell invasion into collagen gels. As CD138 has been shown to have important effects on tumor cell growth, survival, adhesion and invasion, it may be an important regulator in cancer biology.

### 2. PRINCIPLE OF THE METHOD

A capture antibody highly specific for CD138 has been coated to the wells of the microtiter strip plate during manufacture. Binding of CD138 in samples and known standards to the capture antibodies and subsequent binding of the biotinylated anti-CD138 secondary antibody to the analyte is completed during the same incubation period. Any excess unbound analyte and secondary antibody is then removed. The HRP conjugate solution is then added to every well including the zero wells. Following incubation, excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue colored complex with the conjugate. The color development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced colored complex is directly proportional to the concentration of CD138 present in the samples and standards. The absorbance of the color complex is then measured and the generated OD values for each standard are plotted against expected



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concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of CD138 in any sample tested.

### 3. REAGENTS PROVIDED AND RECONSTITUTION

REAGENTS	Quantity (2 x 96 wells)	RECONSTITUTION
CDK064B-P: 96-well microtiter plate	2	Ready-to-use (pre-coated).
CDK064B-Z: Plastic cover	4	Ready-to-use.
CDK064B-A: Standard :16 ng/mL	4 vials	Resuspend each vial in 1.25mL
CDK064B-B: Standard Diluent Buffer	1 vial (25 mL)	10x Concentrate, dilute in distilled water. See Assay preparation.
CDK064B-C: Biotinylated anti-CD138	2 vials (400 µL)	Dilute in Biotinylated Antibody Diluent. See Assay preparation.
CDK064B-D: Biotinylated Antibody Diluent	1 vial (13 mL)	Ready-to-use.
CDK064B-E: Streptavidin-HRP	4 vials (5 µL)	Add 500 µL of HRP diluent prior to use. See Assay preparation.
CDK064B-F: HRP Diluent	1 vial (23 mL)	Ready-to-use.
CDK064B-G: Wash Buffer	2 vials (10 mL)	200x Concentrate dilute in distilled water. See Assay preparation.
CDK064B-H: TMB Substrate	1 vial (24 mL)	Ready-to-use.
CDK064B-I: H <sub>2</sub> SO <sub>4</sub> : Stop Reagent	2 vials (11 mL)	Ready-to-use.

### 4. STORAGE INSTRUCTIONS

Store kit reagents between 2-8 °C. Immediately after use remaining reagents should be returned to cold storage (2-8 °C). Expiration of the kit and reagents is stated on box front label. The expiration of the kit components can only be guaranteed if the components are stored properly, and if the reagent is not contaminated by handling.

**Wash Buffer (CDK064B-G):** Once prepared store at 2-8 °C for up to 1 week.

**Standard Diluent Buffer (CDK064B-B):** Once prepared store at 2-8 °C for up to 1 week.

**Standards (CDK064B-A):** Once prepared use immediately and do not store.

**Biotinylated Antibody (CDK064B-C):** Once prepared use immediately and do not store.

**Streptavidin-HRP (CDK064B-E):** Once prepared use immediately and do not store.

### 5. MATERIALS REQUIRED BUT NOT PROVIDED

- Microtiter plate reader fitted with appropriate filters (450 nm required with optional 630 nm reference filter)



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- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000 µL adjustable single channel micropipettes with disposable tips
- 50-300 µL multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Miscellaneous laboratory plastic and/or glass, if possible sterile

### 6. SPECIMEN COLLECTION, PROCESSING AND STORAGE

Cell culture supernatants, human serum, plasma or other biological samples are suitable for use in the assay.

**Serum:** Remove serum from the clot or red cells as soon as possible after clotting and separation, respectively.

**Cell culture supernatants:** Remove particulates and aggregates by spinning at approximately 1000 x g for 10 minutes.

If not analyzed shortly after collection, samples should be aliquoted (250-500 µL) to avoid repeated freeze-thaw cycles and stored frozen at -80 °C. Avoid multiple freeze-thaw cycles of frozen specimens. Do not thaw by heating at 37 °C or 56 °C. Thaw at room temperature and make sure that the sample is completely thawed and homogeneous before use. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

### 7. SAFETY & PRECAUTIONS FOR USE

- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures, e.g. CDC/NIH Health manual: "Biosafety in Microbiological and Biomedical Laboratories" 2009.
- The human serum included in this kit have been tested and found non-reactive for HBsAg, anti-HIV1 & 2, and anti-HCV. Nevertheless, no known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.
- Laboratory gloves should be worn at all times.
- Avoid any skin contact with H<sub>2</sub>SO<sub>4</sub> and TMB. In case of contact, wash thoroughly with water.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.
- When not in use, kit components should be stored refrigerated or frozen as indicated on vial or bottle labels.
- All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips.
- Cover or cap all reagents when not in use.
- Do not mix or interchange reagents between different lots.
- Do not use reagents beyond the expiration date of the kit.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination. For the dispensing of H<sub>2</sub>SO<sub>4</sub> and substrate solution, avoid pipettes with metal parts.
- Use a clean plastic container to prepare the washing solution.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.



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- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. **Warning TMB is toxic avoid direct contact with hands. Dispose of properly.**
- If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbance's within 1 hour after completion of the assay.
- Maintain a consistent order of addition of reagents to wells to ensure equal incubation times for all wells.
- Dispense the TMB solution within 15 minutes of the washing of the microtiter plate.

### 8. ASSAY PREPARATION

Bring all reagents to room temperature before use.

#### 8.1 Assay Design

Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running zeros and standards. Each sample, standard and zero should be tested **in duplicate**. Remove sufficient Microwell Strips for testing from the aluminum pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. *Seal tightly and return to 2-8 °C storage.*

**Example plate layout** (example shown for a 6 point standard curve):

	Standards		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	16	16										
B	8	8										
C	4	4										
D	2	2										
E	1	1										
F	0.5	0.5										
G	zero	zero										
H												

*All remaining empty wells can be used to test samples in duplicate*

#### 8.2 Preparation of Wash Buffer

Dilute the (200x) wash buffer concentrate 200 fold with distilled water to give a 1x working solution. Pour entire contents (10 mL) of the Washing Buffer Concentrate into a clean 2,000 mL graduated cylinder. Bring final volume to 2,000 mL with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle.

*Store Wash Buffer at 2°-8 °C for up to 1 week.*

#### 8.3 Preparation of Standard Diluent Buffer

Add the contents of the vial (10x concentrate) to 225 mL of distilled water before use.

*Standard Diluent Buffer can be stored at 2-8 °C for up to 1 week.*

#### 8.4 Preparation of Standard

Resuspend **each** vial with 1.25 mL of standard diluent. This reconstitution gives a stock solution of 16 ng/mL of CD138.

**Mix the reconstituted standard gently by inversion only.** Serial dilutions of the standard are made directly in the assay



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plate to provide the concentration range of 16 to 0.5 ng/mL. A fresh standard curve should be produced for each new assay.



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### 8.4 Preparation of Standard (continued)

- Immediately after reconstitution add 200  $\mu$ L of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 16 ng/mL.
- Add 100  $\mu$ L of appropriate standard diluent to the remaining standard wells B1 and B2 to F1 and F2.
- Transfer 100  $\mu$ L from wells A1 and A2 to B1 and B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells.
- Continue this 1:1 dilution using 100  $\mu$ L from wells B1 and B2 through to wells F1 and F2 providing a serial diluted standard curve ranging from 16 ng/ml to 0.5 ng/mL.
- Discard 100  $\mu$ L from the final wells of the standard curve (F1 and F2).

Alternatively, these dilutions can be performed in separate clean tubes and immediately transferred to the relevant wells.

### 8.5 Preparation of Samples

Before testing, serum or plasmas samples have to be diluted 1:2 in standard buffer diluent.

### 8.6 Preparation of Biotinylated anti-CD138

It is recommended this reagent is prepared immediately before use. Dilute the biotinylated anti-CD138 with the biotinylated antibody diluent in an appropriate clean glass vial using volumes appropriate to the number of required wells. Please see example volumes below:

Number of wells required	Biotinylated Antibody ( $\mu$ L)	Biotinylated Antibody Diluent ( $\mu$ L)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

### 8.7 Preparation of Streptavidin-HRP

It is recommended to centrifuge vial for a few seconds in a micro centrifuge to collect all the volume at the bottom.

Dilute the 5  $\mu$ L vial with 500  $\mu$ L of HRP diluent **immediately before use**. Do-not keep this diluted vial for future experiments. Further dilute the HRP solution to volumes appropriate for the number of required wells in a clean glass vial. Please see example volumes below:

Number of wells required	Streptavidin-HRP ( $\mu$ L)	Streptavidin-HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10



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### 9. METHOD

We strongly recommend that every vial is mixed thoroughly without foaming prior to use **EXCEPT** the standard vial, which must be mixed gently by inversion only.

Prepare all reagents as instructed in section 8.

**Note:** final preparation of Biotinylated anti-CD138 (section 8.6) and Streptavidin-HRP (section 8.7) should occur immediately before use.

Assay Step		Details
1.	Preparation	<b>Prepare Standard curve</b> as shown in section 8.4.
2.	Addition	Add 100 µL of each <b>standard, sample and zero</b> in duplicate to appropriate number of wells.
3.	Addition	Add 50 µL of diluted <b>biotinylated anti-CD138</b> to all wells.
4.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18-25 °C) for <b>2 hours</b> .
5.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well. b) Dispense 300 µL of <b>1x washing solution</b> into each well. c) Aspirate the contents of each well. d) Repeat step b and c another two times.
6.	Addition	Add 100 µL of <b>Streptavidin-HRP</b> solution into all wells.
7.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18-25 °C) for <b>30 minutes</b> .
8.	Wash	Repeat wash step 5.
9.	Addition	Add 100 µL of ready-to-use <b>TMB Substrate Solution</b> into all wells.
10.	Incubation	Incubate in the dark for <b>5-15 minutes*</b> at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
11.	Addition	Add 100µL of <b>H<sub>2</sub>SO<sub>4</sub> Stop Reagent</b> into all wells.
<b>Read the absorbance</b> value of each well (immediately after step 11.) on a spectrophotometer using 450 nm as the primary wave length and optionally 630 nm as the reference wave length (610 nm to 650 nm is acceptable).		

*\*Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the colour development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range.*





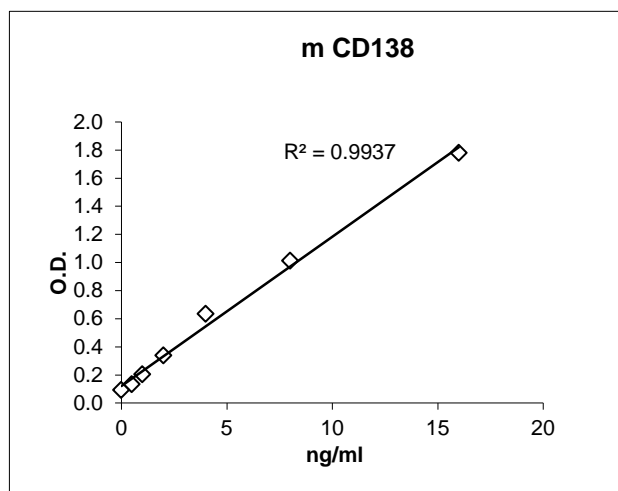
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### 10. DATA ANALYSIS

Calculate the average absorbance values for each set of duplicate standards, controls and samples. Ideally duplicates should be within 20% of the mean. Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding CD138 standard concentration on the horizontal axis. The amount of CD138 in each sample is determined by extrapolating OD values against CD138 standard concentrations using the standard curve. For sample serum or plasmas which have been diluted 1:2 according to the protocol, the calculated concentration should be multiplied by the dilution factor (x2).

Example CD138 Standard curve

Standard	CD138 Concentration (ng/ml)	OD (450 nm) mean	CV (%)
1	16	1.807	3.1
2	8	1.068	6.3
3	4	0.666	0.7
4	2	0.340	6.5
5	1	0.205	2.1
6	0.5	0.134	4.2
Zero	0	0.094	0.8



**Note:** Curve shown above is an example only and should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed.

### 11. ASSAY LIMITATIONS

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples always multiply results by the appropriate dilution factor to produce actual final concentration.

The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Washing Buffer fill with Washing Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.





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As with most biological assays conditions may vary from assay to assay therefore **a fresh standard curve must be prepared and run for every assay.**

### 12. PERFORMANCE CHARACTERISTICS

#### 12.1 Sensitivity

The sensitivity, minimum detectable dose of mouse CD138 using this CD138 ELISA kit was found to be **<0.35 ng/mL**. This was determined by adding 2 standard deviations to the mean OD obtained when the zero standard was assayed 30 times in five independent assays.

#### 12.2 Specificity

The assay recognizes natural and recombinant mouse CD138. To define specificity of this ELISA, several proteins were tested for cross reactivity. There was no cross reactivity observed for any mouse protein tested: mTNF $\alpha$ , mIFN $\gamma$ , mL-2, mGM-CSF, mL-2R, mCD141, mL-10, mL-17A, mL-23. The kit shows a very small level of cross-reactivity (<3.7%) to human CD138.

#### 12.3 Precision

##### Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates (3 duplicates) in 2 mouse pooled serum, 2 in culture media and 2 in standard diluent with samples containing different concentrations of mCD138. 2 standard curves were run on each plate. **The overall intra-assay coefficient of variation has been calculated to be 6.3%.**

Session	Sample	Mean CD138 ng/ml	SD	CV
Session 1	Sample 1	12.08	0.52	4.3
	Sample 2	6.6	0.78	11.8
	Sample 3	12.37	0.27	2.2
	Sample 4	7.23	0.87	12.1
	Sample 5	10.59	0.10	0.9
	Sample 6	8.38	0.74	8.8
Session 2	Sample 1	11.62	0.64	5.5
	Sample 2	7.00	0.06	0.8
	Sample 3	11.53	0.69	6.0
	Sample 4	7.62	0.34	4.4
	Sample 5	9.87	0.64	6.5
	Sample 6	8.21	0.19	2.3
Session 3	Sample 1	11.58	0.29	2.5
	Sample 2	6.33	0.56	8.9
	Sample 3	11.61	0.90	7.8
	Sample 4	6.18	0.81	13.1
	Sample 5	10.07	0.67	6.6
	Sample 6	7.71	0.64	8.3

##### Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments. Each assay was carried out with 6 replicates (3 duplicates) in 2 mouse pooled serum, 2 in culture media and 2 in standard diluent with samples containing different concentrations of CD138. 2 standard curves were run on each plate. **The calculated overall coefficient of variation was 7.6%.**



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	<i>Sample 1</i>	<i>Sample 2</i>	<i>Sample 3</i>	<i>Sample 4</i>	<i>Sample 5</i>	<i>Sample 6</i>
Mean CD138 ng/ml	11.76	6.60	11.84	6.93	10.18	8.09
SD	0.5	0.58	0.71	0.91	0.57	0.62
CV	4.2	8.8	6.0	13.2	5.6	7.6

### 12.4 Dilution Parallelism

Two spiked mouse serum with different levels of recombinant CD138 were analyzed at three serial two fold dilutions (1:2-1:16) with two replicates each. Recoveries ranged from 69% to 136% with an overall **mean recovery of 113%**.

### 12.5 Spike Recovery

The spike recovery was evaluated by spiking three concentrations of recombinant mCD138 in mouse serum and culture media in two experiments. Recoveries ranged from 68% to 103% with an overall **mean recovery of 85% when spiked in serum**. Recoveries ranged from 114% to 126% with an overall **mean recovery of 121% when spiked in culture media**.

### 12.6 Stability

#### Storage Stability

Aliquots of spiked serum sample and culture media were stored at -20 °C, 2-8 °C, room temperature (RT), and 37 °C, and the mCD138 level determined after 24 hours. There was no significant loss of CD138 reactivity during storage.

#### Freeze-thaw Stability

Aliquots of spiked serum were stored frozen at -20 °C and thawed up to 5 times and mCD138 level was determined. There was less than 10% loss after 1 to 5 cycles.

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### 13. ASSAY SUMMARY

Total procedure length: 2 hours 45 minutes.

Add 100 µL of sample and diluted standard/controls and 50 µL  
Biotinylated anti-mouse CD138.



Incubate 2 hours at room temperature.



Wash three times.



Add 100 µL of Streptavidin-HRP.



Incubate 30 minutes at room temperature.



Wash three times



Add 100 µL of ready-to-use TMB.  
Protect from light. Let the color develop for 5-15 minutes.



Add 100 µL H<sub>2</sub>SO<sub>4</sub>.



Read Absorbance at 450 nm.

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