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Human sCD138 (Syndecan-1) ELISA Kit

Catalog No. CDK012A
CDK012B

Quantity: 1 x 96 tests
2 x 96 tests

PRODUCT SPECIFICATIONS :

Specificity :	Recognizes natural human soluble syndecan-1
Range :	8 ng / ml - 256 ng / ml
Sensitivity :	< 2.56 ng / ml
Incubation :	1 h 45 min
Sample Types :	Serum Plasma Cell culture supernatant
Cross Reaction :	No cross reactivity with other human soluble molecules
Kit Content :	Pre-coated 12 strip plate, biotinylated secondary antibody, standards, controls (when available), buffers, Streptavidin-HRP, TMB, Stop Reagent.

1. INTENDED USE

The Cell Sciences® sCD138 ELISA kit is a solid phase sandwich ELISA for the *in-vitro* qualitative and quantitative determination of sCD138 in supernatants, buffered solutions or serum and plasma samples. This assay will recognize both natural and recombinant human sCD138.

This kit has been configured for research only. Not suitable for use in therapeutic procedures.

2. PRINCIPLE OF THE METHOD

A capture Antibody highly specific for CD138 has been coated to the wells of the microtitre strip plate provided during manufacture. Binding of CD138 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 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 concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of CD138 in any sample tested.

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3. REAGENTS PROVIDED AND RECONSTITUTION

Reagents (Store @ 2-8°C)	**Quantity** 1 x 96 well kit	**Reconstitution**
P: 96 well microtiter strip plate	1	Ready to use. (Pre-coated)
A: Plastic plate covers	2	n/a
B: Standard: 256 ng/ml	2	Reconstitute as directed on the vial (see Assay preparation, section 8).
C: Control	2	Reconstitute as directed on the vial (see Assay preparation, section 8).
D: Standard Diluent (Buffer)	1 vial (25 ml)	10x Concentrate, dilute in distilled water (see Assay preparation, section 8)
E: Biotinylated anti-CD138	1 vial (0.4 ml)	Dilute in biotinylated antibody diluent (see Assay preparation, section 8).
F: Streptavidin-HRP	2 vials (5 µl)	Add 0.5 ml of HRP diluent prior to use (see Assay preparation, section 8).
G: HRP Diluent	1 vial (23 ml)	Ready to use.
H: Wash Buffer	1 vial (10 ml)	200x Concentrate, dilute in distilled water (see Assay preparation, section 8).
I: TMB Substrate	1 vial (11 ml)	Ready to use.
J: H_2SO_4 stop reagent	1 vial (11 ml)	Ready to use.

4. MATERIAL REQUIRED BUT NOT PROVIDED

- Microtiter plate reader fitted with appropriate filters (450 nm required with optional 620 nm reference filter)
- 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

5. STORAGE INSTRUCTIONS

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

6. SPECIMEN COLLECTION, PROCESSING & STORAGE

Cell culture supernatants, human serum, plasma, or other biological samples are suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

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

Serum: Avoid any unintentional stimulation of the cells by the procedure. Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. After clotting, centrifuge at approximately 1000 x g for 10 min and remove serum.

Plasma: EDTA, citrate and heparin collected plasma can be assayed. Spin samples at 1000 x g for 30 min. to remove particulates. Harvest plasma.

Storage: 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.

Recommendation: 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 haemolysed or lipemic sera. If large amounts of particles are present, these should be removed prior to use by centrifugation or filtration.

7. SAFETY AND 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" 1984.
- 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_2SO_4 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 from deterioration.
- 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_2SO_4 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.
- 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 absorbances immediately after completion of the assay.

- When pipetting reagents, maintain a consistent order of addition from well to well. This will ensure equal incubation times for all wells.
- Follow incubation times described in the assay procedure.
- Dispense the TMB solution immediately after washing 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 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 U/ml		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	256	256										
B	128	128										
C	64	64										
D	32	32										
E	16	16										
F	8	8										
G	zero	zero										
H	CTRL	CTRL										

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 the final volume to 2,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store 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.
This solution can be stored at 2-8ºC for up to 1 week.

8.4 Preparation of Standard

Standard vials must be reconstituted with the volume of standard diluent shown on the vial immediately prior to use. This reconstitution gives a stock solution of 256 ng/ml of CD138. **Mix the reconstituted standard gently by inversion only**. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 256 to 8 ng/ml. A fresh standard curve should be produced for each new assay.

- Immediately after reconstitution, add 200 µl of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 256 ng/ml.
- Add 100 µl of Standard Diluent to the remaining standard wells B1 and B2 to F1 and F2.
- Transfer 100 µl 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 µl from wells B1 and B2 through to wells F1 and F2, providing a serial diluted standard curve ranging from 256 ng/ml to 8 ng/ml.
- Discard 100 µl from the final wells of the standard curve (F1 and F2).

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

8.5 Preparation of Controls

The supplied Controls must be reconstituted with the volume of Standard Diluent indicated on the vial. Reconstitution of the freeze-dried material with the indicated volume will give a solution at the concentration stated on the vial. Do not store after use.

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 (µl)	Biotinylated Antibody Diluent (µl)
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 microcentrifuge to collect all the volume at the bottom.

Dilute the 5 µl vial with 0.5 ml 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 (µl)	Streptavidin-HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

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 shown in section 8.
Note: Final preparation of Biotinylated anti-IL-4R (section 8.5) and Streptavidin-HRP (section 8.6) should occur immediately before use.

***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 color development within individual micro-wells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range.*

Assay Step		Details
1.	Addition	**Prepare Standard curve** as shown in section 8.4 above.
2.	Addition	Add 100µl of each**, Sample, Standard, Control and zero** in duplicate to appropriate number of wells.
3.	Addition	Add 50µl of diluted **biotinylated anti-CD138** to all wells.
4.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **1 hour.**
5.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well. b) Dispense 0.3 ml of **1x washing solution** into each well. c) Aspirate the contents of each well. d) Repeat step b and c another two times.
6.	Addition	Add 100µl of **Streptavidin-HRP** solution into all wells.
7.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **30 min.**
8.	Wash	Repeat wash step 5.
9.	Addition	Add 100 µl of ready-to-use **TMB Substrate Solution** into all wells.
10.	Incubation	Incubate in the dark for **12-15 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.
11.	Addition	Add 100µl of **H_2SO_4: Stop Reagent** into all wells.
Read the absorbance value of each well (immediately after step 11.) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).		

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10. DATA ANALYSIS

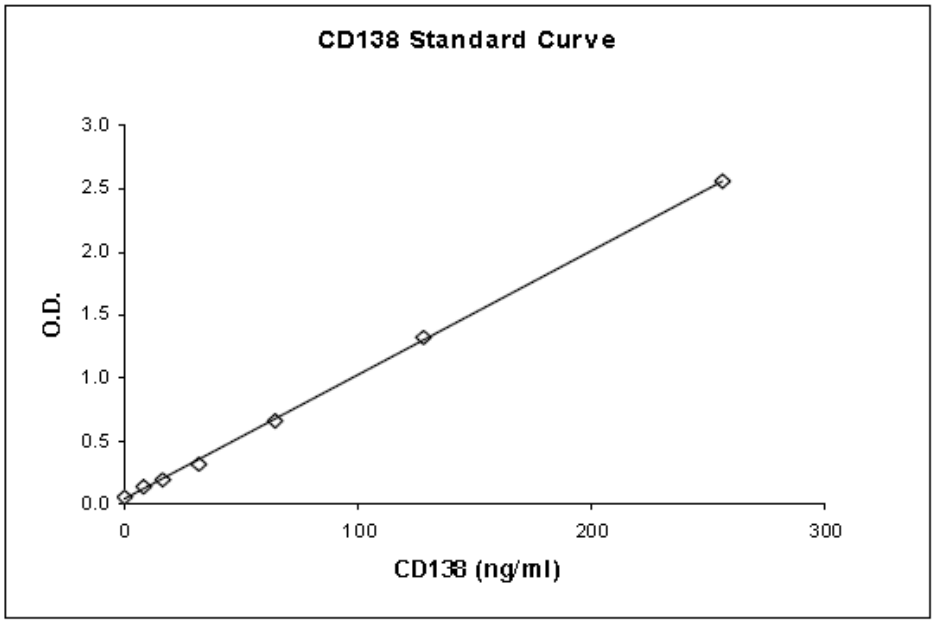
Calculate the average absorbance values for each set of duplicate standards, controls, and samples. Ideally duplicates should be within 20% of the mean.

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**.**

Example sCD138 Standard Curve

Standard	CD138 Concentration pg/ml	OD (450 nm) mean	CV (%)
1	256	2.554	7.4
2	128	1.317	10.8
3	64	0.667	2.0
4	32	0.320	8.8
5	16	0.199	1.4
6	8	0.132	9.1
zero	0	0.053	24.0



Note: The curve shown should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed.

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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 the 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.

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 or minimum detectable dose of CD138 using this Diaclone CD138 ELISA kit was found to be 4.94ng/ml. This was determined by adding 2 standard deviations to the mean OD obtained when the zero standard was assayed in 6 independent experiments.

12.2 Specificity

The assay recognizes both natural and recombinant human CD138. To define the specificity of this ELISA, several proteins were tested for cross reactivity. There was no cross reactivity observed for any protein tested (IL-1b, IL-2 IL-4, IFNgamma, IL-6, IL-6R, TRAIL, IL-7, IL-12 and IL-21).

12.3 Precision

Reproducibility within the assay will be evaluated in three independent experiments. Each assay will be carried out with 6 replicates (3 duplicates) in 2 human pooled serum, 2 in RPMI, and 2 in standard diluent with samples containing different concentrations of CD138. 2 standard curves were run on each plate. **The overall intra-assay coefficient of variation was calculated to be 6.2%.**

Intra-Assay

Session 1

Sample	Mean CD138 ng/ml	SD	CV
1	194.01	17.20	8.9
2	86.13	7.09	8.2
3	195.00	16.10	8.3
4	86.75	7.04	8.1
5	294.13	31.93	10.9
6	174.58	15.30	8.8

Session 2

Sample	Mean CD138 ng/ml	SD	CV
1	171.65	3.94	2.3
2	79.97	5.85	7.3
3	173.60	5.80	3.3
4	76.61	2.42	3.2
5	242.90	15.80	6.5
6	169.50	7.72	4.6

Session 3

Sample	Mean CD138 ng/ml	SD	CV
1	183.07	11.48	6.3
2	90.91	4.18	4.6
3	191.82	10.77	5.6
4	93.35	3.92	4.2
5	264.00	14.66	5.6
6	158.20	8.64	5.5

Inter-Assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by two technicians. Each assay was carried out with 6 replicates (3 duplicates) in 2 human pooled serum, 2 in RPMI, 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 10.2%.**

Sample	1	2	3	4	5	6
Mean CD138 ng/ml	197	88	199	88	274	162
SD	21	8	19	9	27	19
CV	10.5	9.5	9.4	10.0	9.9	12.0

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12.4 Dilution Parallelism

In two independent experiments, two spiked human serum samples with different levels of CD138 were analyzed at different serial two fold dilutions (1:2 to 1:8) with two replicates each. Recoveries ranged from 59 to 122% with an overall **mean recovery of 85%.**

12.5 Spike Recovery

The spike recovery was evaluated by spiking 3 concentrations of CD138 in human serum and culture medium in 3 separate experiments. Recoveries ranged from 87 to 113% with an overall **mean recovery of 101%**

12.6 Stability

Storage Stability

Aliquots of spiked serum and spiked medium were stored at –20°C, 4°C, room temperature (RT) and at 37°C and the CD138 level determined after 24h. There was no significant loss of CD138 reactivity during storage at 4°C, RT, or 37°C.

Freeze-thaw Stability

Aliquots of spiked serum and spiked medium were stored frozen at –20°C and thawed up to 5 times. The CD138 level was determined. There was no significant loss of CD138 reactivity after 5 cycles of freezing and thawing.

12.7 Expected Serum Values

A panel of 40 human sera and 40 Plasma samples were tested for CD138. See results below:

Sample Matrix	Number of Samples Evaluated	Range (ng/ml)	Mean (ng/ml)	Standard Deviation (ng/ml)
Serum	40	16.17-205.16	48.15	36.5
Plasma	40	15.7-68.9	31.62	15.3

13. ASSAY SUMMARY

Total procedure length: 1h 45 min

Add 100 µl of sample and diluted standard/controls and 50 µl Biotinylated anti-CD138.
↓
Incubate 1 hour at room temperature.
↓
Wash three times.
↓
Add 100 µl of Streptavidin-HRP.
↓
Incubate 30 min at room temperature.
↓
Wash three times.
↓
Add 100µl of ready-to-use TMB
↓
Protect from light. Let the color develop for 12-15 min.
↓
Add 100 µl of H_2SO_4.
↓
Read the Absorbance at 450 nm.

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