Human sCD138 (Syndecan-1) ELISA Kit

Instructions for use

Catalogue numbers:	1x48 tests: EA102169 1x96 tests: EA102170 2x96 tests: EA102171

For research use only

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Human sCD138 (Syndecan-1) ELISA KIT

1. Intended use

The OriGene 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 recognise both natural and recombinant human sCD138.

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

2. Introduction

2.1. Summary

Syndecans are a transmembrane protein family within the heparin sulphate proteoglycan group that interact with many different molecules of the immune system through their heparin sulphate chains. The mammalian syndecan family consists of 4 proteins; syndecan 1 to 4 each encoded by very distinct genes. In adult tissues syndecan 1 (CD138) is predominantly expressed by epithelial cells and plasma cells (both normal and malignant) and currently considered 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. Syndecan 1 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.
Via its heparin sulphate chains CD138 binds to and modulates the activity of a wide range of molecules involve in inflammation including chemokines, growth factors, selectins and other adhesion molecules. CD138 can also act as a receptor for collagen, fibronectin, thrombospondin and tenascin therefore involved in cell matrix adhesion. CD138 has been shown to mediate the binding of myeloma cells to type I collagen, and inhibits tumour cell invasion into collagen gels.

As CD138 has been shown to have important effects on tumour cell growth, survival, adhesion and invasion syndecan-1 may be an important regulator in cancer biology.

2.2. Principle of the method

A capture Antibody highly specific for CD138 has been coated to the wells of the microtiter 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 coloured complex with the conjugate. The colour development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced coloured complex is directly proportional to the concentration of CD138 present in the samples and standards. The absorbance of the colour 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.

3. Reagents provided and reconstitution

Reagents (Store@2-8°C)	**Quantity 1x48 well kit** Cat no. EA102169	**Quantity 1x96 well kit** Cat no. EA102170	**Quantity 2x96 well kit** Cat no. EA102171	**Reconstitution**
96 well microtiter strip plate	1/2	1	2	Ready to use (Pre-coated)
Plastic plate covers	2	2	4	n/a
Standard: 256ng/ml	1	2	4	Reconstitute as directed on the vial (see reagent preparation, section 8)
Standard Diluent (Buffer)	1 (25ml)	1 (25ml)	1 (25ml)	10x Concentrate, dilute in distilled water (see reagent preparation, section 8)
Control	1	2	4	Reconstitute as directed on the vial (see reagent preparation, section 8)
Biotinylated anti-CD138	1 (0.4ml)	1 (0.4ml)	2 (0.4ml)	Dilute in biotinylated antibody diluent (see reagent preparation, section 8)
Biotinylated Antibody diluent	1 (7ml)	1 (7ml)	1 (13ml)	Ready to use
Streptavidin-HRP	1 (5µl)	2 (5µl)	4 (5µl)	Add 0.5ml of HRP diluent prior to use (see reagent preparation, section 8)
HRP Diluent	1 (23ml)	1 (23ml)	1 (23ml)	Ready to use
Wash Buffer	1 (10ml)	1 (10ml)	2 (10ml)	200x Concentrate dilute in distilled water (see reagent preparation, section 8)
TMB Substrate	1 (11ml)	1 (11ml)	1 (24ml)	Ready to use
H_2SO_4 stop reagent	1 (11ml)	1 (11ml)	2 (11ml)	Ready to use

4. Materials required but not provided

- Microtiter plate reader fitted with appropriate filters (450nm required with optional 620nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000µl adjustable single channel micropipettes with disposable tips
- 50-300µl 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 kit reagents between 2 and 8°C. Immediately after use remaining reagents should be returned to cold storage (2-8°C). Expiry of the kit and reagents is stated on box front labels. The expiry 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 will be 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: Use pyrogen/endotoxin free collecting tubes. Serum should be removed rapidly and carefully from the red cells after clotting. Following clotting, centrifuge at approximately 1000 x g for 10 min and remove serum.

Plasma: EDTA, citrate and heparin 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 (250-500µl) to avoid repeated freeze-thaw cycles and stored frozen at –70°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 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 & 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.
- 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 vials or bottles 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 colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly.
- If a dark blue colour 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.
- 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 within 15 min 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, zero and control 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^{o}C storage.

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

	Standards / Controls		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 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˚-25˚C.

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 2-8°C 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 256ng/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 8ng/ml. A fresh standard curve should be produced for each new assay.

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

Alternatively these dilutions can be performed in separate clean tubes and immediately transferred 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 5µl vial with 0.5ml 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.

Prepare all reagents as shown in section 8.
Note: final preparation of Biotinylated Secondary Antibody (section 8.6) and Streptavidin-HRP (section 8.7) should occur immediately before use.

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 (Standard diluent)** 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 100µl 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).		

***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.*

10. Data Analysis

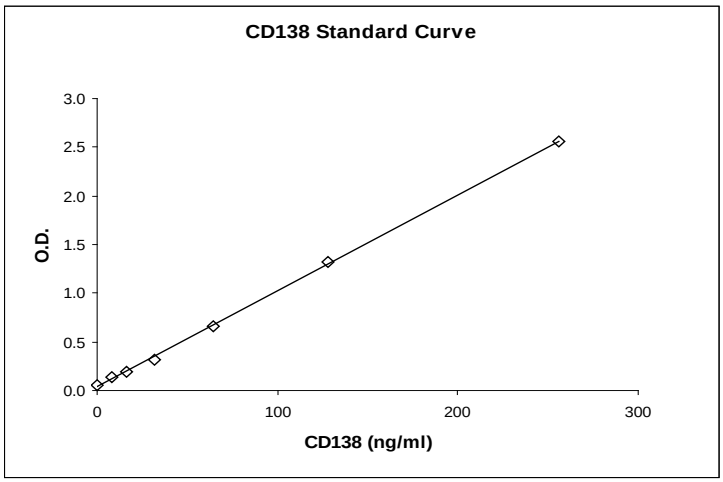
Calculate the average absorbance values for each set of duplicate standards, controls and samples. Ideally duplicates should be within 20% of the mean.

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 Conc	OD (450nm) 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: curve shown above 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.

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 OriGeneCD138 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-1β, IL-2 IL-4, IFNγ, IL-6, IL-6R, TRAIL, IL-7, IL-12 and IL-21).

12.3. Precision

Intra-assay

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 has been calculated to be 6.2%.**

Session	***Sample***	**Mean** CD138 ng/ml	**SD**	**CV**
Session 1	***Sample 1***	194.01	17.20	**8.9**
	Sample 2	86.13	7.09	**8.2**
	Sample 3	195.00	16.10	**8.3**
	Sample 4	86.75	7.04	**8.1**
	Sample 5	294.13	31.93	**10.9**
	Sample 6	174.58	15.30	**8.8**
Session 2	***Sample 1***	171.65	3.94	**2.3**
	Sample 2	79.97	5.85	**7.3**
	Sample 3	173.60	5.80	**3.3**
	Sample 4	76.61	2.42	**3.2**
	Sample 5	242.90	15.80	**6.5**
	Sample 6	169.50	7.72	**4.6**
Session 3	***Sample 1***	183.07	11.48	**6.3**
	Sample 2	90.91	4.18	**4.6**
	Sample 3	191.82	10.77	**5.6**
	Sample 4	93.35	3.92	**4.2**
	Sample 5	264.00	14.66	**5.6**
	Sample 6	158.20	8.64	**5.5**

Inter-assay

Assay to assay reproducibility within one laboratory will be evaluated in three independent experiments by two technicians. 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 calculated overall coefficient of variation was 10.2%.**

	Sample 1	***Sample 2***	***Sample 3***	***Sample 4***	***Sample 5***	***Sample 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**

12.4. Dilution Parallelism

In two independent experiments two spiked human serum samples with different levels of CD138 were analysed 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 and 37°C.

Freeze-thaw Stability
Aliquots of spiked serum and spiked medium were stored frozen at –20°C and thawed up to 5 times and 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. Bibliography

Bartlett A et.al. Molecular and cellular mechanisms of syndecans in tissue injury and inflammation. Mol. Cells, Vol 24, No. 2. pp. 153-166. 2007
Gotte et.al. Syndecan-1 as a regulator of chemokine function. The Scientific world, 3, pp. 1327-1331. 2003
Gotte et.al. Syndecans in Inflammation. FASEB, 17, pp. 575-591. 2003
Masouleh et al. Role of the Heparin Sulphate Proteoglycan Syndecan-1 (CD138) in Delayed type Hypersensitivity. Journal of immunology, 182, pp. 4985-4993. 2009
Wanping Sun et.al. A Novel Anti-Human Syndecan-1(CD138) Monoclonal Antibody 4B3: Characterization and Application. Cellular & Molecular Immunology, 209, Volume 4, 2007.
Wijdenes J., Clement C., Klein B, Dore J-M. (1997). CD138 (syndecan-1) Workshop Panel report.
Wijdenes J., Dore JM., Clement C., Vermot-Desroches C.(2002) CD138
J.Biol.Regul.Homeost. Agents 16: 152-155.

14. OriGeneHuman CD138 ELISA references

Adepu, S. et al., Am J Physiol Renal Physiol.,2015; 309(2): F137-145
Andersen N.F. et al.,Br J Haematol.,2005;128:210-217
Annecke, T. et al., Br. J. Anaesth.,2010;104(4): 414-421
Bruegger D. et al., Am J Physiol Heart Circ Physiol, 2005; 289(5):H1993-9
Bruegger, D. et al., J. Thorac. Cardiovasc. Surg.,2009;138(6): 1445-1447
Celie, J. W. A. M. et al., Am J Physiol Renal Physiol.,2008; 294(1): F253-263.
Chappell, D. et al.,Cardiovasc. Res.,2009;83(2):388-396
Cigliana, G. et al.,J Exp Clin Cancer Res., 2015;34(1):37
Connolly-Andersen, A.-M. et al.,Open Forum Infect Dis.,2014; 1(1): ofu027-
Hofmann-Kiefer, K. F. et al., Reproductive Sciences, 2013; 20:318-325
Iwata, H. et al., Haematologica,2004; 89(3): 368-370.
Janosi, J. et al., Haematologica,2004; 89(3): 370-371.
Jilani I. et al.,Int J Lab Hematol.,2009; 31:97-105
Joensuu H. et al., Cancer Res., 2002; 62 : 5833 - 5842
Kliment,C.R. et al.,J Biol. Chem., 2009 ; 284(6):3537-3545
Kristensen, I.B. et al., Blood (ASH Annual Meeting Abstracts),2012;120:3977
Kyrtsonis M-C. et al., Blood, 2004; 104(11): 4882
Kyrtsonis, M.-C. et al.,Blood (ASH Annual Meeting Abstracts),2005;106(11): 3404.
Lovell R. et al.,Br J Haematol.,2005; 130:542-548
Maisnar V. et al., Neoplasma.2006;53:26-29
Mahtouk, K. et al.,Blood,2007;109: 4914 - 4923
Meuwese, M. C. et al., J. Lipid Res.,2008; P800025-JLR200.
Molica, S. et al.,Leuk Lymphoma, 2006; 47(6): 1034-40.
Nault, J.-C. et al., Cancer Epidemiol. Biomarkers Prev.,2013; 22(8): 1343-1352.
Rehm, M. et al., Circulation,2007; 116(17): 1896-1906.
Schaar, C. G. et al., Haematologica,2005; 90(10): 1437-1438.
Seidel C. et al., Blood, 2000; 95 : 388 - 392
Snoeijs, M. G. et al., Am J Physiol Renal Physiol.,2010; 299(5): F1134-1140
Szarvas T. et al.,Hum Pathol.,2014;45:674-682
Theocharis, A. D. et al.,J Biol Chem.,2006; 281(46): 35116-28.
Thiara, A. S. et al, Perfusion,2010; 25(1): 9-16
Thiara, A. S. et al., Perfusion,2011; 26(2): 107-114
Vassilakopoulos T.P.et al.,Anticancer Res.,2005; 25:4743-4746
Vlahu, C. A. et al.,J. Am. Soc. Nephrol., 2012; 23: 1900-1908
Wang X. et al., Br J Cancer.,2014;111:1965-1976

15. Assay Summary

Total procedure length: 1h45mn

Add 100 µl of sample and diluted standard/controls and 50µl Biotinylated anti-CD138

↓

Incubate 1 hour at room temperature

↓

Wash three times

↓

Add 100µl of Streptavidin-HRP

↓

Incubate 30min at room temperature

↓

Wash three times

↓

Add 100µl of ready-to-use TMB
Protect from light. Let the color develop for 12-15 mn.

↓

Add 100µl H_2SO_4

↓

Read Absorbance at 450 nm

TECHNICAL CONSULTATION

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Not for use in diagnostic procedures

16. International Summaries

16.1. French

PREPARATION DES REACTIFS : RESUME

1. Tampon de Lavage *(Washing Buffer)*

Ajouter 10 ml de **Tampon de Lavage concentré** (*Washing Buffer Concentrate*) 200 fois (200X) à 1990 ml d'eau distillée

2 Tampon de Dilution du Standard *(Standard Diluent Buffer)*

Ajouter 25 ml de **Tampon de Dilution du Standard concentré** 10 fois (*Standard Diluent Buffer Concentrate* 10X*)* à 225 ml d'eau distillée

3. Standard CD138 *(CD138 Standard)*

Reconstituer le **Standard CD138** en ajoutant la quantité indiquée sur le flacon de Tampon de Dilution du Standard

4. Contrôles *(CD138 Controls)*

Reconstituer les **Contrôles** (*Controls*) en ajoutant la quantité indiquée sur le flacon de Tampon de Dilution du Standard

5. Anti-CD138 Biotinylé *(Biotinylated anti-sCD138)*

Nombre de barrettes	**Anti-CD138 Biotinylé Concentré (µl)**	**Diluent de l'Anticorps Biotinylé** (µl)
2	40	1060
3	60	1590
4	80	2120
6	120	3180
12	240	6360

6. Streptavidin-HRP (*Streptavidin-HRP*)

Nombre de barrettes	**Streptavidin-HRP pré-diluée** (µl)	**Diluent HRP** (ml)
2	30	2
3	45	3
4	60	4
6	75	5
12	150	10

RESUME DU PROTOCOLE OPERATOIRE: durée totale : 1h45mn

1. Ajouter 100 µl de **Tampon de Dilution du Standard** (*Standard Diluent Buffer*), en duplicat, dans les puits Standards (B1 à F2).
2. Ajouter à la pipette 200 µl de **Standard CD138** *(CD138 Standard)* reconstitué dans les puits A1 et A2 puis réaliser des dilutions du Standard allant de 256 à 8 ng/ml en transférant 100 µl d'un puits à l'autre. Jeter les 100 µl des derniers puits (F1 et F2).
3. Ajouter 100 µl de **Tampon de Dilution du Standard** *(Standard Diluent Buffer)* en duplicat dans les puits "blancs".
4. Ajouter 100 µl d'**échantillon** *(Sample)*, en duplicat, dans les puits désignés et 100 µl de contrôle *(CD138 control),* en duplicat dans les puits contrôles.
5. Préparer l'**anticorps anti-CD138 Biotinylé** *(Biotinylated anti CD138).*
6. Ajouter 50 µl d'anticorps **anti-CD138 Biotinylé dilué** *(diluted biotinylated anti CD138)* dans tous les puits.
7. Couvrir les barrettes de puits et incuber pendant 1 heure à température ambiante (18-25°C).
8. Vider et laver les puits 3 fois avec le **Tampon de Lavage** *(Washing Buffer).*
9. Préparer la Streptavidin-HRP.
10. Ajouter 100 µl de **Streptavidin-HRP diluée** *(diluted HRP-Streptavidin)* dans tous les puits.
11. Couvrir les puits et incuber pendant 30 minutes à température ambiante (18-25°C).
12. Vider et laver les puits 3 fois avec le **Tampon de Lavage** *(Washing Buffer).*
13. Ajouter 100 µl de solution de TMB *(TMB solution)* prête à l'emploi dans tous les puits y compris les "blancs".
14. Incuber pendant environ 10-15 minutes à température ambiante (18-25°C) à l'obscurité.
15. Ajouter 100 µl d'H_2SO_4: **Solution Stop** *(H_2SO_4 : Stop Solution)* dans tous les puits y compris les "blancs".
16. Mesurer l'absorbance (Densité Optique = D.O.) à la longueur d'onde 450 nm et optionnellement à 620 nm (entre 610 et 650 nm) comme longueur d'onde de référence.

Remarque: Les échantillons présentant une valeur de D.O. excédant la gamme de la courbe Standard peuvent résulter à des taux de CD138 incorrects. C'est pourquoi, il est recommandé de diluer de tels échantillons avec le Tampon de Dilution du Standard *(Standard Diluent Buffer)* **afin de quantifier précisément le véritable taux de CD138.**

16.2. Spanish

PREPARACIÓN DE LOS PRODUCTOS

1. Tampón de Lavado *(Washing Buffer)*	Añadir **Tampón de Lavado Concentrado** 200 X (10 ml) *(Washing Buffer Concentrate)* a 1990 ml de agua destilada.		
2 Tampón diluyente del estándar *(Standard Diluent Buffer)*	Añadir **Tampón Diluyente del Estándar Concentrado** 10 X (25 ml) *(Standard diluent buffer concentrate 10X)* a 225 ml de agua destilada.		
3. Estándar CD138 *(CD138 Standard)*	Reconstituir el **Estándar CD138** añadiendo el Diluyente del Estándar, como indica la etiqueta del vial.		
4. Controles CD138 *(CD138 Control)*	Reconstituir los **controles** añadiendo el Diluyente del Estándar, como indica la etiqueta del vial.		
5. Anti-CD138 biotinilado *(Biotinylated anti-CD138)*	Número de tiras	**Anticuerpo biotinilado concentrado** (µl)	**Diluyente del anticuerpo biotinilado** (µl)
	2	40	1,060
	3	60	1,590
	4	80	2,120
	6	120	3,180
	12	240	6,360
6. Estreptavidina-HRP *(Streptavidin-HRP)*	Número de tiras	**Estreptavidina-HRP prediluida** (µl)	**Diluyente de HRP** (ml)
	2	30	2
	3	45	3
	4	60	4
	6	75	5
	12	150	10

RESUMEN DEL PROTOCOLO. El procedimiento total tiene una duración de 1h45min.

1. Añadir 100 µl del **Tampón Diluyente del Estándar** *(Standard Diluent Buffer Concentrate 10X)*, por duplicado, a los pocillos designados para el estándar (B1 to F2).
2. Pipetear 200 µl del **Estándar CD138** *(CD138 Standard)* reconstituido en los pocillos A1 y A2 y hacer diluciones seriadas del estándar con el rango de concentraciones de 256 al 8 ng/ml, transfiriendo 100 µl de un pocillo al siguiente. Descartar 100 µl de los últimos pocillos.
3. Añadir 100 µl del **Tampón Diluyente del Estándar**, por duplicado, a los pocillos que van a ser el "blanco".
4. Añadir 100 µl de las muestras, por duplicado, a los pocillos designados para ello, y 100 µl del Control reconstituido *(CD138 Control)*, por duplicado, a los pocillos designados como "control".
5. Preparar el anticuerpo **Anti-CD138 Biotinilado** *(Biotinylated anti-sCD138).*
6. Añadir 50 µl del **anti-CD138 Biotinilado** y diluido, a todos los pocillos.
7. Cubrir la placa e incubar durante 1 horas a temperatura ambiente (18-25°C).
8. Vaciar y lavar la placa 3 veces con **Tampón de Lavado** *(Washing Buffer).*
9. Preparar la **Estreptavidina-HRP** *(Streptavidin-HRP).*
10. Añadir 100 µl de **Estreptavidina-HRP** diluida a todos los pocillos.
11. Cubrir la placa e incubar 30 minutos a temperatura ambiente (18-25°C).
12. Vaciar y lavar la placa 3 veces con **Tampón de Lavado**.
13. Añadir 100 µl de solución **TMB preparado para utilizar** *(TMB Substrate)*, a todos los pocillos, incluidos los pocillos con "blancos".
14. Incubar la placa durante 10-15 minutos a temperatura ambiente (18-25°C) y en oscuridad.
15. Añadir 100 µl de H_2SO_4: **Solución de Parada** *(H_2SO_4 Stop Solution)*, a todos los pocillos, incluidos los pocillos con los "blancos".
16. Medir la intensidad de color (densidad óptica) a 450 nm y a 620 nm como longitud de onda de referencia (de 610 nm a 650 nm sería aceptable).

Nota: El cálculo de concentraciones de muestras con densidad óptica que supere el rango de la curva estándar, resultaría incorrecto, dando niveles de CD138 más bajos de lo real. Estas muestras, requerirían ser diluidas con el Tampón de Dilución de Estándar, para poder precisar la cantidad real de CD138.