HUMAN SOLUBLE CD112 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE CD112 CONCENTRATIONS IN SERUM AND PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN SOLUBLE CD112 ELISA**
Catalog No.	SK00663-01
Lot No.	
Formulation	96 T
Standard Range	23.4 ~ 1500 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL per well
Sample Type	Serum, plasma
Specificity	Human soluble CD112
Calibration	Human CD112 recombinant, extracellular domain
Sample Dilution	10-20 **(Optimal dilutions should be determined by each laboratory for each application)**
Intra-assay Precision	6 - 8%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

INTRODUCTION

This Human Soluble CD112/Nectin-2 ELISA Kit contains the necessary components required for the quantitative measurement of natural and recombinant human CD112 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human CD112 and antibody raised against this protein. Results from this immunoassay have shown to accurately quantify natural and recombinant human CD112 samples.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human CD112 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human CD112 present is bound by the immobilized antibody. After washing away any unbound substances, antibody-HRP conjugate specific for human CD112 is added to the wells. Following a wash to remove any unbound enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of human CD112 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
CD112 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against human CD112.	**663-01-01**	**1 plate**
CD112 Standard – 3000 pg/vial of recombinant human CD112 in a buffered protein base with preservative; lyophilized.	**663-01-02**	**1 vial**
Detection Antibody-HRP Conjugate – 110 µL/vial, 100-fold concentrated of Antibody-HRP conjugate against human CD112 with preservative.	**663-01-03**	**1 vial**
Positive Control - one vial of human CD112; lyophilized.	**663-01-04**	**1 vial**
Dilution Buffer - 60 mL of buffered protein based solution with preservative.	**DB10**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 8 months. For longer storage, unopened Standard and Positive Control should be stored at -20° C or -70° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples will require dilution. A pretest is needed to optimize the dilution of samples. A 10-fold dilution is suggested. To make a 10-fold dilution is 30 µL sample + 270 µL Dilution Buffer. A 20-fold dilution is suggested. To make a 10-fold dilution is 15 µL sample + 285 µL Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application.

Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer – If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer.

CD112 Standard – Reconstitute the CD112 standard with 1.0 mL of Dilution Buffer. The concentration of the reconstituted stock solution is 3000 pg/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	3000 pg/ml
# 1	250 µl of stock	250 µl	1500 pg/ml
# 2	250 µl of 1	250 µl	750 pg/ml
# 3	250 µl of 2	250 µl	375 pg/ml
# 4	250 µl of 3	250 µl	187.5 pg/ml
# 5	250 µl of 4	250 µl	93.75 pg/ml
# 6	250 µl of 5	250 µl	46.875 pg/ml
# 7	250 µl of 6	250 µl	23.4 pg/ml

Positive Control - Reconstitute the Positive Control with 1.0 mL Dilution Buffer.

Detection Antibody-HRP Conjugate - Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light)**. **DO NOT FREEZE.**

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents, standard dilutions, positive control and samples as directed previously.
2. Add 100 µL per well of **Dilution Buffer** to Blank wells.
3. Add 100 µL per well of **Standard dilutions** in reverse order of serial dilution from #7 - 1, **samples**, or **positive control**. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
4. Aspirate and wash each well with 300 µL of **1x Wash Buffer** four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).

5. Add 100 µL per well of **Detection Antibody-HRP Conjugate working solution**. Cover with plate sealer and incubate for 1 hour on microplate shaker at room temperature. **Protect from light.**
6. Repeat the aspiration and wash as in step 5.
7. Add 100 µL per well of **Substrate Solution**. Incubate for 17-20 minutes on microplate shaker at room temperature. **Protect from light.**
8. Add 100 µL per well of **Stop Solution**. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Read plate using a microplate reader set to 450 nm within 15 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	CORRECTED (450NM)
Blank	0 (0.120)
23.4	0.040
46.8	0.094
93.75	0.211
187.5	0.369
375	0.728
750	1.407
1500	2.667

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human sCD112	100
Human sCD36	0
Human sCD146	0
Human sCD14	0
Human sCD163	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS
↓
Add 100 µL of standard dilutions, samples or positive control. Cover with plate sealer and incubate 2 hours on microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Detection Antibody-HRP conjugate working solution. Cover with plate sealer and incubate 1 hour on microplate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Substrate Solution. Incubate 17-20 min on microplate shaker at RT. **Protect from light.**
↓
Add 100 µL per well of Stop Solution. Read at 450 nm within 15 minutes.