HUMAN SOLUBLE CD320 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF CD320 CONCENTRATIONS IN RAT AND MOUSE SERUM AND EDTA PLASMA

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN SOLUBLE CD320 ELISA KIT
Catalog No.	SK00209-01
Lot No.	
Formulation	96 T
Standard range	0.32 ~ 200 ng/ml
Dynamic range	1.6 ~200 ng/ml
Sensitivity	0.3 ng/ml
Sample Volume	50 µl per well
Dilution Factor	*Optimal dilutions should be determined by each laboratory for each application*
Sample Type	Serum, EDTA plasma
Specificity	Human soluble CD320
Intra-assay Precision	6-8%
Inter-assay Precision	12-14%
Storage	4 °C

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INTRODUCTION

Human soluble CD320 ELISA employs the quantitatively competitive enzyme immunoassay technique in which human CD320 present in samples competed with a fixed amount of biotinylated Rat CD320 for sites on purified rabbit IgG specific against human CD320. During the incubation, the rabbit IgG becomes bound to the goat anti-rabbit IgG pre-coated onto the microplates. Following a wash to remove any unbound antibody, standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of human CD320 bound in the initial step. The sample values are then read off the standard curve.

Human soluble CD320 ELSA has been shown to accurately quantitate the recombinant and natural human CD320. Results obtained using natural Rat CD320 showed dose response curves that were parallel to the standard curves obtained using the kit standards.

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.
_Some vials contain small quantities of material, therefore centrifuge before use.

Precautions for Use

All reagents should be considered as potentially hazardous. The stop solution contains diluted Hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.

We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
R-Microplate - 96 well microplate pre-coated with polyclonal anti rabbit IgG Fc purified IgG	**RM01**	**1 plate**
CD320 Standard -200 ng/vial of recombinant Rat CD320 in a buffered protein base with preservatives; lyophilized	**209-01-01**	**1 vial**
Biotin Solution Concentrated - 350 µL/vial, 10-fold concentrated of Rat CD320 biotinylated with preservatives; lyophilized	**209-01-02**	**1 vial**
CD320 Antibody Concentrated – 350 µl/vial, 10-fold concentrated of polyclonal purified IgG against Rat CD320 with preservatives; lyophilized	**209-01-03**	**1 vial**
Positive Control – one vial of recombinant Rat CD320 , lyophilized (optional)	**209-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
HRP Diluent Solution - 12 mL/vial of buffered protein based solution with preservatives	**DB06C**	**1 vial**
Dilution Buffer - 60mL/vial of buffered protein based solution with preservatives. Ready to use.	**DB18**	**1 vial**
Wash Buffer - 50 ml/vial, 10-fold concentrated buffered surfactant, with preservative	**WB01**	**1 vial**
TMB Substrate Solution - 11 ml/vial of TMB substrate	**TMB01**	**1 vial**

solution		
Stop Solution - 11 ml/vial of contains 0.5 M HCl	**S-STOP**	**1 vial**
Plate Sealer	**EAPS**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Biotin Solution Concentrated should be stored at -20 or -70 °C for up to 8 months. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (1000 ng/ml), Biotin Solution , Positive Control and Antibody SHOULD BE STORED at -20 °C or – 70°C for up to one months. Reconstituted Biotin Solution (350 µl) CAN NOT BE STORED at 2-8°C. Streptavidin - HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

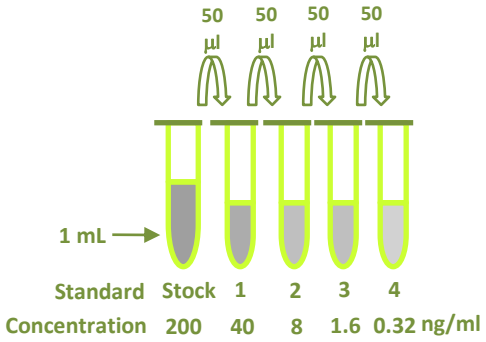
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

CD320 Standard - Refer to vial label for reconstitution volume. Reconstitute the **CD320** Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 200 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 µL of Dilution Buffer into tubes #1 to #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 ng/mL standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	1 ml	200 ng/ml
# 1	50µl of stock	200µl	40 ng/ml
# 2	50µl of 1	200µl	8 ng/ml
# 3	50µl of 2	200µl	1.6 ng/ml
# 4	50µl of 3	200µl	0.32 ng/ml



Antibody - Reconstitute the **Antibody Concentrate** with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 3.15 mL of Dilution Buffer to prepare **1x Antibody Solution**.

Biotin Solution - Reconstitute the **Biotin Solution Concentrate** with 350 µl of Dilution Buffer to make 10-fold concentrated solution. Transfer it to 3.15 mL of Dilution Buffer to prepare **1x Biotin Solution**.

Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 12 mL of **HRP Diluent Solution** to prepare working solution. **Note**: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. *Positive Control should be prepared and used immediately*.

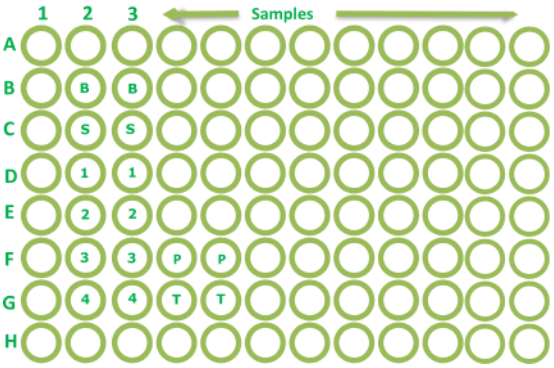
ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards and PC be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic bag containing the desiccant pack, reseal.
3. Leave well D4 and D5 as Blank. ***DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.***
4. Set G4 and G5 as total binding. Add 50 µl per well of **Dilution Buffer**.
5. Add 50 µl per well of **standard solution** from #4 to #1 (reverse order of serial dilution) to the appropriate wells (C2, to G3). Add 50 µl per well of **Positive Control** into wells F4 and F5. Add 50 µl per well of **samples** into appropriate wells.
6. Add 25µl per well of **1x Antibody Solution** into total binding, standard, PC and samples wells. Cover or seal the plate and incubate on Microplate shaker (250-300rpm) at room temperature for 2 hours. ***Note: DO NOT ASPIRATE AND WASH PLATE***. ***PROCEED IMMEDIATELY TO THE NEXT STEP.***
7. Add 25 µl per well of **1x Biotin Solution** into total binding, standard, PC and samples wells. Cover or seal the plate and incubate at room temperature for 2 hours. **Note:** DO NOT ADD Biotin Solution to Blank wells.
8. Aspirate wells and wash 5 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
9. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate on microplate shaker for one hour at room temperature.
10. Aspirate and wash as step 8.
11. Add 100 µL of **Substrate Solution** to each well. Incubate for 3-8 minutes at room temperature. **Protect from light**.
12. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. It is recommended to add the stop solution when the total Binding or the lowest standard has developed a dark blue color.
13. Determine the optical density of each well within 15 minutes. Set the microplate reader to to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate. Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, PC, and samples and subtract the average Blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



TYPICAL DATA

These standard curves * are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human sCD320.

SENSITIVITY

300 pg/ml

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human sCD320,	100%
Human sCD36	0
Human sCD209	0
Human sRAGE	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 50µl of standard, samples, positive control to each well. Add 25 µL of 1X Antibody solution to each well. Incubate 2 hours on the plate shaker at RT. DO NOT ASPIRATE AND WASH PLATE. PROCEED IMMEDIATELY TO THE NEXT STEP.
↓
Add 25 µl 1X Biotin Solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 5 times.
↓
Add 100 µl Streptavidin HRP conjugate working solution to all wells. Incubate 1 hour on the plate shaker at RT.
↓
Aspirate and wash 5 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 3-8 min on the bench top. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min