HUMAN SOLUBLE CD320/ TRANSCOBALAMIN RECEPTOR (TCBIR) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE CD320/TCBIR CONCENTRATIONS IN URINE, SERUM AND EDTA PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITENS OF EACH KIT BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN SOLUBLE CD320 /TCBIR ELISA KIT**
Catalog No.	SK00209-01C
Lot No.	20114820
Formulation	96 T
Standard range	125 ~ 8000 pg/mL
Sensitivity	20 pg/mL
Sample Volume	100 µL
Sample Type	Urine, Serum, EDTA Plasma
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Specificity	Human soluble CD320/TCBIR
Calibration	Human soluble CD320-Fc fusion recombinant (NS0 derived)
Intra-assay Precision	4 - 6%
Inter-assay Precision	5 - 9%
Storage	2 – 8° C for 6 months. More information check page 2-3
This kit contains sufficient materials to run approximately 35-40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human Soluble CD320/Transcobalamin Receptor (TCBIR) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural soluble human TCBIR from urine, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human soluble TCBIR-Fc fusion and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural soluble TCBIR samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human sTCBIR. The capture antibody can bind to the human sTCBIR in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human sTCBIR is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human sTCBIR bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix 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.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Human sTCBIR /TCBIR Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human sTCBIR.	**209-01-01**	**1 plate**
Human sTCBIR Standard – 8000 pg/vial of recombinant human sTCBIR-Fc fusion (NS0 derived) in a buffered protein base with preservative; lyophilized.	**209-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against human TCBIR with preservative; lyophilized.	**209-01-03**	**1 vial**
Positive Control - one vial of recombinant human TCBIR-Fc; lyophilized.	**209-01A-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB05**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB11C**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08B**	**1 bottle**
Wash Buffer – 25 mL of 20-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 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 4 months. For long-term storage up to 10 months, place unopened Standard, Positive Control, and Detection Antibody Concentrate, Dilution Buffer and Antibody & HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2 – 8° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (**200-250 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) 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, heparin, or citrate 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

Serum or EDTA plasma samples do not need to be diluted. Urine samples may need to be diluted by 10 ~ 20 fold.
Optimal dilutions; however, 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 25 mL of Wash Buffer Concentrate 20X into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

Human sTCBIR-Fc Standard - Reconstitute the human sTCBIR standard with 1.0 mL of **Dilution Buffer (DB05)**. This reconstitution produces a stock solution of 8000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer (DB05) into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **8000 pg/mL** standard serves as the high standard. The **Dilution Buffer (DB05)** serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1.0 ml	8000 pg/ml
# 1	250 µl of stock	250 µl	4000 pg/ml
# 2	250 µl of 1	250 µl	2000 pg/ml
# 3	250 µl of 2	250 µl	1000 pg/ml
# 4	250 µl of 3	250 µl	500 pg/ml
# 5	250 µl of 4	250 µl	250 pg/ml
# 6	250 µl of 5	250 µl	125 pg/ml

Positive Control - Reconstitute the Positive Control with 1.0 mL of **Dilution Buffer (DB05)**.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB11C)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody Diluent Solution (DB11C)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working

solution. **Protect from light.** The working solution of Streptavidin-HRP Conjugate should be freshly prepared and used within 2 hours.

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 and working standards as directed in the previous sections.
2. Add 100 µL of **Dilution Buffer (DB05)** to Blank wells.
3. Add 100 µL of **Standard dilutions** in reverse order of serial dilution, **samples**, or **positive control** per well. Cover with plate sealer. Incubate for **2 hours** on microplate shaker (400-450 rpm) at room temperature. Optional incubating for 14 hours at 2-8 °C.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for 25-30 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 3 min.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log or 4-Parameter curve fit.

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

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Soluble TCBIR-Fc (NS0 derived)	100
Human Soluble TCBIR His Tag (E. Coli)	100
Mouse TCBIR (HEK293)	0
Human Transcobalamin (HEK293)	0
Human IgG1 Fc Recombinant (HEK293)	0

TYPICAL DATA

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

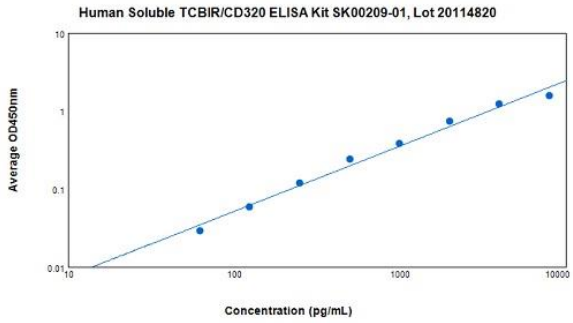
STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.099)
62.5 Optional	0.029
125	0.059
250	0.119
500	0.240
1000	0.379
2000	0.725
4000	1.209
8000	1.537

- Lot: 20114820
- Positive control: 300 - 1200 pg/mL

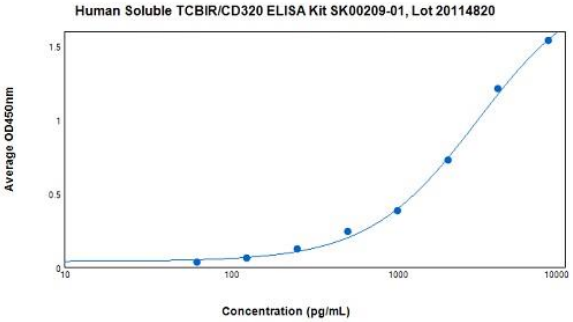
SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker (200-250 rpm) at RT. Optional incubating for 14 hours at 2-8 °C.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 25 - 30 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 3 min.

Standard Curve by Log-log fit:



Standard curve by 4-parameter fit:



The research human serum or EDTA plasma samples were diluted by Dilution Buffer DB05. Its linearity and recovery was assayed by Human sCD320 ELISA Kit SK00209-01**.

Sample	Dilution Factor	Assayed (pg/mL)	Final (pg/mL)	Recovery (%)
Plasma	2 X	298.125	596.250	100
Plasma	4 X	147.964	591.856	99
Serum	2 X	384.824	769.648	100
Serum	4 X	198.445	793.778	103
Urine*	10 X	302.159	3021.591	100
Urine*	20 X	183.662	3673.241	121

*urine sample was freshly collected and non-centrifuged. Urine sample was diluted by Dilution Buffer DB05.

Citation: