HUMAN CTRP3 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN CTRP3 CONCENTRATIONS IN SERUM AND PLASMA

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN CTRP3 ELISA
Catalog No.	SK00082-07
Lot No.	
Formulation	96 T
Standard Range	5-320 ng/mL
Sensitivity	2 ng/mL
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma
Dilution factor	**Optimal dilutions should be determined by each laboratory for each application**
Specificity	Human CTRP3
Calibration	Human CTRP3 (46-246) Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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.

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DESCRIPTION

This Human CTRP3 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human CTRP3 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human CTRP3 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural CTRP3 samples. Due bovine CTRP3 was identical to human CTRP3 (99.1%), do not use any fetal bovine serum or other animal serum in assay matrix.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an monoclonal antibody specific for human CTRP3. The capture antibody can bind to the human CTRP3 in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against human CTRP3 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 CTRP3 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.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_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.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
CTRP3 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against CTRP3.	082-07-01	1 plate
CTRP3 Standard – refer to lot specific of recombinant human CTRP3 in a buffered protein base with preservative; lyophilized.	082-07-02	1 vial
Detection Antibody Concentrate –refer to lot specific of an antibody against CTRP3 with preservative; lyophilized.	082-07-03	1 vial
Positive Control - one vial of recombinant human CTRP3; lyophilized.	082-07-04	1 vial
Streptavidin-HRP Conjugate refer to lot specific of Goat anti rabbit IgG conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 30 mL of buffered protein based solution with preservative.	DB06	1 bottle
Antibody & HRP Diluent Solution – 30 mL of buffered protein based solution with preservative.	DB68C	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, Positive Control and Detection Antibody Concentrate should

be stored at -20° C or -70° C. Do not use kit past expiration date.
Streptavidin HRP Conjugate concentrated solution and TMB Substrate Solution can be stored at 2 – 8° C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**).
Microplate Wells: Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

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) 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. *Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.*

Optimal dilutions should be determined by each laboratory for each application.
Due bovine CTRP3 was identical to human CTRP3 (99.1%), do not use any fetal bovine serum or other animal serum in assay matrix. For cell culture sample assay, use animal free media only.

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 1x Wash Buffer.

CTRP3 Standard - Reconstitute the CTRP3 standard with refer to lot specific. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **320 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFER	CONCENTRATION
Stock	Powder	Refer to lot	
# 1	Refer to lot	Refer to lot	320 ng/ml
# 2	250 µl of 1	250 µl	160 ng/ml
# 3	250 µl of 2	250 µl	80 ng/ml
# 4	250 µl of 3	250 µl	40 ng/ml
# 5	250 µl of 4	250 µl	20 ng/ml
# 6	250 µl of 5	250 µl	10 ng/ml
# 7	250 µl of 6	250 µl	5 ng/ml

Positive Control - Reconstitute the positive control with refer to lot specific of Dilution Buffer to make positive control solution.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with refer to lot specific of **Antibody & HRP Diluent Solution** to produce a 10-fold concentrated stock solution. Pipette 10.8 mL of **Antibody & HRP Diluent Solution** into a 15 mL centrifuge tube and transfer 1.2 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin Conjugate - Pipette refer to lot specific mL of Dilution Buffer into a 15 mL centrifuge tube and transfer refer to lot specific µl of concentrated stock solution to prepare working solution. **Note: (protect from light)**.

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. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of Dilution Buffer to Blank wells.
4. Add 100 µL of standard solutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. 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.
6. 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.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot specific at room temperature on microplate shaker. **Protect from light.**
11. 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.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

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 curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the CTRP3 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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 (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.064)
5	0.048
10	0.080
20	0.170
40	0.345
80	0.743
160	1.403
320	2.636

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human CTRP3 (46-246)	100%
Human CTRP1, globular form	0
Human CTRP9, globular form	0
Human adiponectin, globular form	0

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 at RT.

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 a plate shaker at RT. **Protect from light.**

Aspirate and wash 4 times.

Add 100 µl Substrate solution to each well. Incubate for refer to lot specific on plate shaker at RT. **Protect from light.**

Add 100 µl Stop Solution to each well. Read 450nm within 15 min.