MOUSE/RAT CTRP6/C1QTNF6 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE/RAT CTRP6/C1QTNF6 CONCENTRATIONS IN SERUM AND PLASMA

THIS STANDARD CURVE IS PROVIDED FOR DEMONSTRATION ONLY. ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS OF EACH KIT 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	**MOUSE/RAT CTRP6 ELISA**
Catalog No.	**SK00089-08**
Formulation	**96 T**
Lot No.	
Standard Range	**19.531 – 5000 pg/mL**
Sensitivity	**5-7 pg/mL**
Sample Volume	**100 µl per well**
Sample Type	**Serum and EDTA Plasma**
Specificity	**Mouse and Rat CTRP6**
Calibrator	**Mouse CTRP6 Full Length Rec**
Sample Dilution	**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 for 1 month. See page 2 for detail**
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

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INTRODUCTION

Mouse/Rat CTRP6 immunoassay is a solid phase ELISA designed to measure mouse or rat CTRP6 in serum and EDTA plasma. It contains recombinant mouse CTRP6 and antibodies raised against this protein. It has been shown to accurately quantify mouse or rat CTRP6. Results obtained with naturally occurring CTRP6 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for mouse or rat CTRP6.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An IgG specific for CTRP6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CTRP6 present is bound by the immobilized IgG. After washing away any unbound substances, a biotinylated IgG specific for CTRP6 is added to the wells. After washing away any unbound biotinylated IgG, a Streptavidin-HRP conjugate 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 CTRP6 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.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
CTRP6 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an IgG against CTRP6.	**089-08-01**	**1 plate**
CTRP6 Standard – refer to lot of CTRP6 in a buffered protein base with preservative; lyophilized.	**089-08-02**	**1 vial**
Detection Concentrate – refer to lot, 10-fold concentrate of biotinylated IgG against CTRP6 with preservative; lyophilized.	**089-08-03**	**1 vial**
Positive Control – one vial of CTRP6; lyophilized.	**089-08-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL of 100-fold concentrated solution of Streptavidin HRP Conjugate.	**SAHRP**	**1 vial**
Dilution Buffer - 40 mL of buffered protein based solution with preservative.	**DB28**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB08B**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
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 1 month. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20 °C or -70 °C. Streptavidin-HRP Conjugate 100-fold concentrated solution **(protect from light)** and TMB Substrate Solution should be only stored at 2-8 °C for up to 10 months. Do not use kit past expiration date.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 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

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.

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.

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

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.

Mouse CTRP6 Standard - Refer to vial label for reconstitution volume. Reconstitute the mouse **CTRP6** standard with refer to lot of Dilution Buffer (DB28). This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 300 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 **5000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	Refer to lot	5000 pg/ml
# 1	100 µl of stock	300 µl	1250 pg/ml
# 2	100 µl of 1	300 µl	312.5 pg/ml
# 3	100 µl of 2	300 µl	78.125 pg/ml
# 4	100 µl of 3	300 µl	19.53 pg/ml

Positive Control – Reconstitute the Positive Control with refer to lot of Dilution Buffer (DB28) to prepare 1x working solution.

Detection Concentrate – Reconstitute the Detection Concentrate with refer to lot of Dilution Buffer (DB28) to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer 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 **Dilution Buffer (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** Streptavidin-HRP conjugate working solution should be used within a few hours **(protect from light)**.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

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 of Dilution Buffer to Blank wells (G1 and G2).
4. Add 100 µL of Standard solutions in reverse order of serial dilutions from #4-#1 (F1, F2 to C1 and C2), Standard stock to B1 and B2, sample, or positive control (G3, G4) per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
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 Concentrate 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-HRP 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 on microplate shaker at room temperature. **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 3 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 (OD vs Log Concentration) 4-Paramenter 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 CTRP6 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.

CALIBRATION

This immunoassay is calibrated against a highly purified mouse CTRP6 recombinant.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Mouse/Rat CTRP6	100
Human CTRP6	25
Human/Mouse CTRP13	0
Mouse CTRP1	0
Mouse CTRP3	0
Mouse CTRP9	0
Mouse CTRP12	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)	CORRECTED (450NM)
Blank	0 (refer to lot)
19.531	0.101
78.125	0.351
312.5	1.122
1250	2.547
5000	3.024

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl of Detection Concentrate working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl of 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 of Substrate Solution to each well. Incubate refer to lot on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl of Stop Solution to each well. Read 450nm within 3 minutes.