MOUSE RAT CTRP9 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE OR RAT CTRP9 CONCENTRATIONS IN SERUM

PURCHASE INFORMATION:

ELISA NAME	MOUSE RAT CTRP9 ELISA
Catalog No.	SK00081-08
Lot No.	
Formulation	96 T
Standard range	78-5000 ng/mL
Sensitivity	500 pg/ml
Sample Volume	100 µl
Sample Type	Serum
Pretreatment	May be needed
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Mouse , Rat
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2 °C-8 °C

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

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INTRODUCTION

Mouse Rat CTRP9 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure mouse CTRP9 in serum. It contains recombinant mouse CTRP9 and antibodies raised against this protein. It has been shown to accurately quantify recombinant mouse CTRP9. Results obtained with naturally occurring CTRP9 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 natural mouse CTRP9.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CTRP9 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CTRP9 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for CTRP9 is added to the wells. Following a wash to remove any unbound antibody reagent, Streptavidin HRP Conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of CTRP9 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ 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
CTRP9 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against CTRP9.	**081-08-01**	**1 plate**
CTRP9 Standard – 5000 ng/vial of recombinant human CTRP9 in a buffered protein base with preservatives; lyophilized.	**081-08-02**	**1 vial**
Detection Antibody Concentrate – 1.05mL/vial, 10-fold concentrated of an antibody against CTRP9 with preservatives; lyophilized.	**081-08-03**	**1 vial**
Positive Control- one vial of recombinant mouse CTRP9, lyophilized	**081-08-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer – 60mL of buffered protein based solution with preservatives	**DB06**	**1 bottle**
HRP Diluent Solution - 12mL of buffered protein based solution with preservatives	**DB01**	**1 bottle**
Pretreatment Solution- 20mL of buffered based solution	**DB81**	**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 piece**
Plastic Pouch	**P01**	**1 piece**

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 or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Antibody Concentrate Solution SHOULD BE STORED at -20 °C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrate **(protect from light)** and other components may be stored at 2 - 8°C for up to 8months. *Diluted standard working solution and positive control should be prepared and used immediately*.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

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.

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.

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.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Samples may need to be treated before being added to the microplate.

Note: pooled mouse serum samples with pretreatment or without pretreatment were tested. The results indicate that both samples could be detectable.

***Standard and Positive Control DO NOT NEED to be treated.**

1. Add 150 µl of DTT (from a fresh stock of 1M) or 300 µl TCEP (from a fresh stock of 500 mM) to 15 mL Pretreatment Solution to reach a final concentration of 10mM to obtain 1x Pretreatment Solution. **Note**: 1) 1M DTT stock or 500mM stock solution must be freshly prepared in deionized or distilled water just prior to usage. **[DTT and TCEP are not included in this kit]** 2) The 1x Pretreatment Solution is not stable and cannot be stored!
2. Add 60 µL of sample to 240 µL of 1x Pretreatment Solution in a polypropylene tube**.** Vortex gently and incubate for 30~45 minutes at room temperature. Assay immediately and discard any excess pretreated sample. ***(This dilution may require optimization.)***

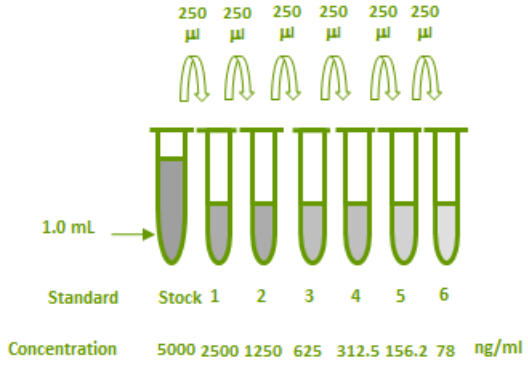
REAGENT PREPARATION

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.

CTRP9 Standard - Refer to vial label for reconstitution volume. Reconstitute the **CTRP9** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 5000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 ng/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	Powder	1000 µl	5000 ng/ml
# 1	250 µl of stock	250 µl	2500 ng/ml
# 2	250 µl of 1	250 µl	1250 ng/ml
# 3	250 µl of 2	250 µl	625 ng/ml
# 4	250 µl of 3	250 µl	312.5 ng/ml
# 5	250 µl of 4	250 µl	156.2 ng/ml
# 6	250 µl of 5	250 µl	78 ng/ml



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of the appropriate Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05mL of 10-fold concentrated stock solution to prepare working solution.

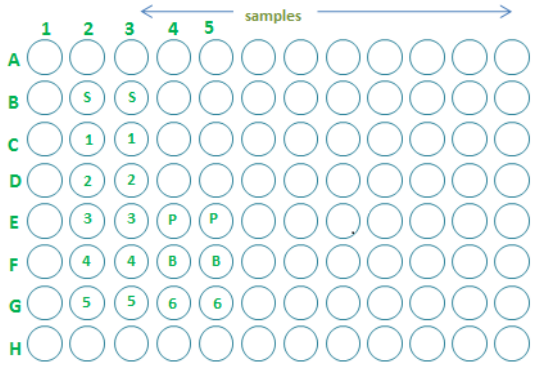
Streptavidin-HRP Conjugate - Pipette 11.94 mL of **HRP Diluent Solution (DB01)** into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days **(protect from light).**

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

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 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 pouch with the desiccant pack and seal.
3. Add 100 µL of Dilution Buffer to Blank wells (F4, F5).
4. Add 100 µL of Standard (B2, B3 to G2, G3 and G4, G5), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate 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 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 micro-plate 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 micro-plate 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 8-12 minutes 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 15 minutes, using a micro-plate reader set to 450nm.



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 CTRP9 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.

Samples which have been treated are diluted by 5, so the concentration read from the standard curve must be multiplied by the dilution factor of 5 (or whatever the optimal dilution factor is.)

TYPICAL DATA

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

STANDARD (NG/ML)	CORRECTED (450NM)
Blank	0 (0.176)
78	0.053
156.2	0.085
312.5	0.226
625	0.397
1250	0.885
2500	1.260
5000	2.101

- Lot No.:
- Positive Control: 200 – 500 ng/mL

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant MOUSE CTRP9.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of CTRP9 was 500 pg/ml

SPECIFICITY

This assay recognizes both natural and recombinant mouse CTRP9. The factors listed below were prepared at 50000 ng/mL in Dilution Buffer, and assayed for cross reactivity.

Proteins	Cross-reactivity
Mouse CTRP9, globular form	100%
Human CTRP1, globular form	0
Human CTRP3, globular form	0
Mouse CTRP3, globular form	0
Mouse adiponectin, globular form	0

Rat serum samples were tested with this kit. The data also indicated that rat serum samples were specifically bound to antibody that was used in this kit formulation condition. Its linear dilution curves were parallel to the standard curves obtained using the ELISA standard. That means rat serum samples cross-react with mouse CTRP9 ELISA kit.

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 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 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 8-12 min on plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min