MOUSE/RAT CTRP12 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE OR RAT CTRP12 CONCENTRATIONS IN SERUM AND PLASMA.

PURCHASE INFORMATION:

ELISA NAME	MOUSE/RAT CTRP12 ELISA
Catalog No.	SK00392-08
Formulation	96 T
Standard Range	0.156-20 ng/mL
Sensitivity	50 pg/mL
Sample Volume	100 µl per well
Sample Type	Serum, EDTA Plasma
Specificity	Mouse, Rat CTRP12
Sample Dilution	
Intra-assay Precision	6-8%
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 CTRP12 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure mouse CTRP12 in serum, and plasma. It contains recombinant mouse CTRP12 and antibodies raised against this protein. It has been shown to accurately quantitie mouse CTRP12. Results obtained with naturally occurring CTRP12 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 CTRP12.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse CTRP12 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CTRP12 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for mouse CTRP12 is added to the wells. Following a wash to remove any unbound antibody reagent. After washing away any unbound antibody, 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 CTRP12 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
CTRP12 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against mouse CTRP12.	392-08-01	1 plate
CTRP12 Standard – 320 ng/vial of mouse CTRP12 in a buffered protein base with preservatives; lyophilized.	392-08-02	1 vial
Detection Antibody Concentrated – 105 µL / vial, 100-fold concentrated of biotinylated Antiobdy against mouse CTRP12 with preservatives;	392-08-03	1 vial
Positive Control- one of mouse CTRP12, lyophilized	392-08-04	1 vial
Streptavidin HRP Conjugate- 120µL of 100-fold concentrated Streptavidin HRP Conjugate	SAHRP	1 vial
Dilution Buffer- 60 mL/vial of buffered protein based solution with preservatives	DB08	1 vial
HRP Diluent Solution- 12 mL/vial of buffered protein based solution with preservatives	DB01A	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
Substrate Solution-11 ml / vial of TMB substrate solution	TMB01	1 vial
Stop Solution -11 ml /vial of 0.5M HCl	S-STOP	1 vial
Plate Sealer.	EAPS	1

STORAGE

Unopened Kit: Store at 2 - 8° C. Do not use past kit expiration date.

Opened / Reconstituted Reagents: Reconstituted Detection Antibody and Standard Stock may be stored for up to 1 month at -70°C. Streptavidin HRP conjugate 100 fold concentrated should be stored at 2 - 8° C .
Microplate Wells: Return unused wells to the plastic zip bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month 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

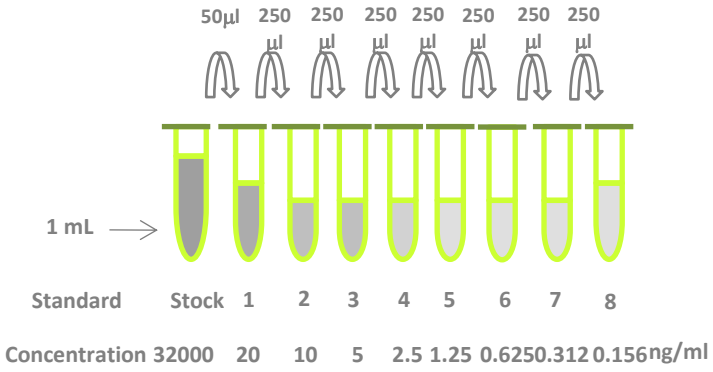
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as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

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.

CTRP12 Standard - Refer to vial label for reconstitution volume. Reconstitute the **CTRP12** Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 320 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 750µL of the appropriate Dilution Buffer into the tube #1 . Pipette 250µL of the appropriate Dilution Buffer into the tube #2 to #8. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20 ng/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

STANDARD	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	320 ng/ml
# 1	50 µl of stock	750 µl	20 ng/ml
# 2	250 µl of 1	250 µl	10 ng/ml
# 3	250 µl of 2	250 µl	5 ng/ml
# 4	250 µl of 3	250 µl	2.5 ng/ml
# 5	250 µl of 4	250 µl	1.25 ng/ml
# 6	250 µl of 5	250 µl	0.625 ng/ml
# 7	250 µl of 6	250 µl	0.312 ng/ml
# 8	250 µl of 7	250 µl	0.156 ng/ml



Detection Antibody – Reconstitute the Detection Antibody concentrated with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of the appropriate Dilution Buffer into the 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 the appropriate HRP Diluent Solution into the 15 ml centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution.

Positive Control –Reconstitute the Positive Control with 1 mL of Dilution Buffer to prepare working solution.

ASSAY PROCEDURE

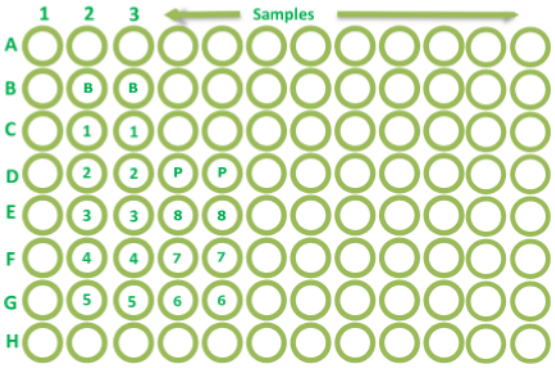
Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

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 foil pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (B2, B3).
4. Add 100 µL of Standard (from C2, C3 to G2, G3, G4, G5 to E4, E5), sample, or control per well (D4, D5). Cover with the 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 (300µL) 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 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 working solution to each well. Incubate for 60 minutes at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 4-7 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.
10. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, 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 CTRP12 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 CTRP12.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of CTRP12 was 50 pg/mL.

TYPICAL DATA

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

STANDARD (NG/ML)	CORRECTED (450NM)
Blank	0 (0.100)
0.156	0.021
0.312	0.045
0.625	0.081
1.25	0.187
2.5	0.330
5	0.616
10	1.015
20	1.495

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl 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 Antibody 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 working solution to each well. Incubate 60 minutes in a paper box (Protect from light) on the plate shaker at RT
↓
Aspirate and wash 4 times.
↓
Add 100 µl of Substrate to each well. Incubate 4-7 min on the bench top. Protect from light.
↓
Add 100 µl of Stop Solution to each well. Read 450nm within 15 min

SPECIFICITY

Mouse CTRP12 ELISA kit recognizes recombinant and endogenous mouse CTRP12. The data also indicated that rat serum samples cross-react with mouse CTRP12 ELISA kit.

PROTEINS	CROSS-REACTIVITY (%)
Mouse CTRP12	100
Human CTRP12	60
Mouse CTRP13	0
Mouse CTRP15	0
Mouse CTRP10	0
Mouse CTRP9	0
Mouse Adiponectin	0