MOUSE/RAT CTRP15 /MYONECTIN ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE/RAT CTRP15/MYONECTIN CONCENTRATIONS IN SERUM AND PLASMA

PURCHASE INFORMATION:

ELISA NAME	MOUSE/RAT CTRP15/MYONECTIN ELISA
Catalog No.	SK00393-08
Lot No.	
Formulation	96 T
Standard Range	0.0312 ~20 ng/mL
Sensitivity	30 pg/mL
Sample Volume	100 µl per well
Sample Type	Serum, EDTA Plasma
Specificity	Mouse and Rat CTRP15/myonectin
Dilution Factor	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 RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

ORDER CONTACT:
AVISCERA BIOSCIENCE, INC.
2348 WALSH AVE., SUITE C
SANTA CLARA, CA 95051
USA
TEL: (408) 982 0300
Email: Info@AvisceraBioscience.com
Website: www.AvisceraBioscience.com

INTRODUCTION

Mouse/Rat CTRP15/ Myonectin immunoassay is a solid phase ELISA designed to measure mouse and rat CTRP15 in serum and plasma. It contains recombinant mouse CTRP15 and antibodies raised against this protein. It has been shown to accurately quantify mouse and rat CTRP15. Results obtained with naturally occurring CTRP15 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 and rat CTRP15.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse CTRP15 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CTRP15 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for mouse CTRP15 is added to the wells. Following a wash to remove any unbound antibody reagent, 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 CTRP15 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
CTRP15 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against CTRP15.	393-08-01	1 plate
CTRP15 Standard – 40 ng/vial of mouse CTRP15 in a buffered protein base with preservative; lyophilized.	393-08-02	1 vial
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against CTRP15 with preservative; lyophilized.	393-08-03	1 vial
Positive Control - one of mouse CTRP15; lyophilized.	393-08-04	1 vial
Streptavidin HRP Conjugate - 120 µL of 100-fold concentrated Streptavidin-HRP Conjugate.	SAHRP	1 vial
Dilution Buffer - 60 mL of buffered protein based solution with preservative.	DB08	1 bottle
HRP Diluent Solution- 12 mL of buffered protein based solution with preservative.	DB06	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 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 °C or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard (stock) and Detection Antibody concentrated solution SHOULD BE STORED at -20 °C or -70 °C for up to one month. Streptavidin-HRP

Conjugate 100-fold concentrated solution **(protect from light)** and other components may be stored at 2 – 8 °C for up to 8 months. Do not freeze TMB substrate solution.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. 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.
- 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.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

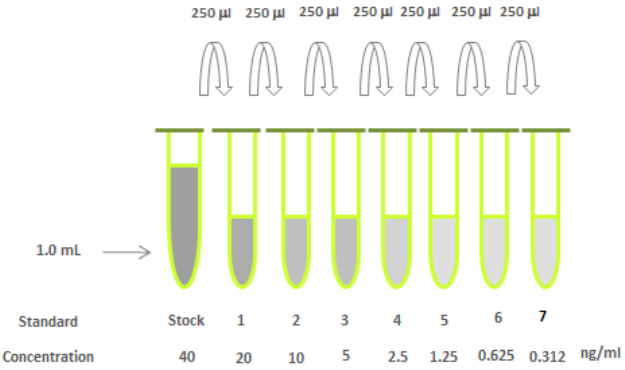
Serum and plasma samples may not require dilution.
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

CTRP15 Standard - Refer to vial label for reconstitution volume. Reconstitute the **CTRP15** standard with 1 mL of **Dilution Buffer**. This reconstitution produces a stock solution of 40 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 #7. 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).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	40 ng/ml
# 1	250 µl of stock	250 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	0.3125 ng/ml



Detection Antibody Concentrate – Reconstitute the Detection Antibody Concentrate 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 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** into a 15 mL centrifuge tube and transfer 120 µL of 100-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 to prepare working solution. **Note**: Positive Control solution could be reused within a few days if stored at -20 °C or -70 °C.

ASSAY PROCEDURE

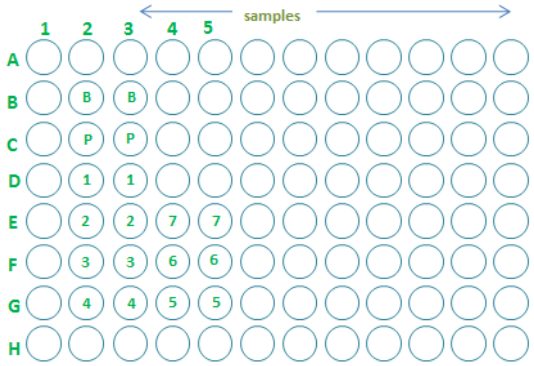
Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess 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 (B2, B3).
4. Add 100 µL of Standard solutions in reverse order of serial dilution (from E4, E5 to G4, G5, G2, G3 to D2, D3), sample, or positive control (C2, C3) 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 (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 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 working solution to each well. Cover with plate sealer. 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 3-7 minutes 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 15 minutes, using a microplate 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 CTRP15 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 purified recombinant mouse CTRP15.

SENSITIVITY

The minimum detectable dose (MDD) of CTRP15 was 30 pg/mL.

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 (0.119)
0.156 (optional)	0.035
0.313	0.078
0.625	0.169
1.25	0.281
2.5	0.492
5	0.908
10	1.470
20	2.187

- Lot No.:
- Positive Control: 1-3 ng/mL

SPECIFICITY

This assay recognizes both natural and recombinant mouse CTRP 15. The data also indicated that rat serum and plasma samples were competitively 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 and plasma samples cross-react with mouse CTRP15 ELISA kit.

PROTEINS	CROSS-REACTIVITY(%)
Mouse CTRP 15	100
Mouse CTRP12	0
Mouse CTRP1	0
Mouse CTRP6	0
Mouse CTRP9	0
Mouse CTRP13	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

↓

Add 100 µl of standard, samples, positive control to the 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 on the plate shaker at RT. **Protect from light.**

↓

Aspirate and wash 4 times.

↓

Add 100 µl of Substrate Solution to each well. Incubate 3-7 min on the plate shaker at RT. **Protect from light.**

↓

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