CTRP9 (MOUSE/RAT) ELISA KIT

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	CTRP9 (MOUSE/RAT) ELISA KIT
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, Plasma
Pretreatment	May be needed
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Specificity	Mouse and Rat
Calibration	Mouse CTRP9 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C for 1 month, see page 2-3 for detail
This kit contains sufficient materials to run approximately 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 Mouse/Rat CTRP9 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural mouse or rat CTRP9 from serum in a sandwich ELISA format.

This immunoassay contains recombinant mouse CTRP9 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural CTRP9 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for mouse CTRP9. The capture antibody can bind to the mouse CTRP9 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against mouse CTRP9 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 is added to the wells and color develops in direct proportion to the amount of mouse CTRP9 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.
_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.

COMPONENTS 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 mouse CTRP9 in a buffered protein base with preservative; lyophilized.	**081-08-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrate of an antibody against CTRP9 with preservative; lyophilized.	**081-08-03**	**1 vial**
Positive Control - one vial of recombinant mouse CTRP9; lyophilized.	**081-08-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
Antibody and HRP Diluent Solution – 30 mL of buffered protein based solution with preservative.	**DB08-B**	**1 bottle**
Pretreatment Solution – 20 mL of buffered based solution.	**STB01**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
Substrate Solution - 11 mL of 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 1 month. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and Antibody & HRP Diluent Solution should be stored at -20° C.

Streptavidin-HRP Conjugate and Substrate Solution should be stored only at 2-8 °C.Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (350 – 400 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.
- 500mM TCEP (fresh preparation) Soltec Ventures, Product #: M115

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.

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

SAMPLE PREPARATION

Due to the polymers of CTRP9 in circulating samples, Samples may need to be treated before assay.

Note: Pooled mouse serum samples with pretreatment or without pretreatment were tested and detectable in this assay.

***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 TCEP 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) 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 minutes at room temperature. Assay immediately and discard any excess pretreated sample. ***(This dilution may require optimization.)***

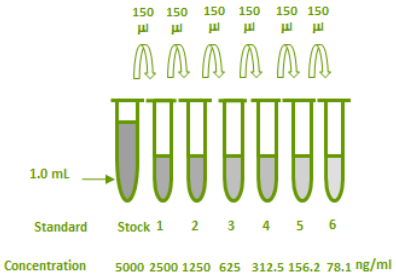
REAGENT PREPARATION

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 1x Wash Buffer.

CTRP9 Standard - 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 150 µL of 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 Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	5000 ng/ml
# 1	150 µl of stock	150 µl	2500 ng/ml
# 2	150 µl of 1	150 µl	1250 ng/ml
# 3	150 µl of 2	150 µl	625 ng/ml
# 4	150 µl of 3	150 µl	312.5 ng/ml
# 5	150 µl of 4	150 µl	156.25 ng/ml
# 6	150 µl of 5	150 µl	78.125 ng/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Antibody and HRP Diluent Solution (DB08-B)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of the **Antibody and HRP Diluent Solution** 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 **Antibody and HRP Diluent Solution (DB08-B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution **(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. Add **50 µL** per well of Dilution Buffer to Blank wells.
3. Add **50 µL** of Standard dilutions, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
4. 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.
5. 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.
6. Repeat the aspiration/wash as in step 4.
7. 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.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 8-12 minutes at room temperature on micro-plate shaker. **Protect from light.**
10. 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.
11. Determine the optical density of each well using a micro-plate reader set to 450nm within 3 minutes.

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 or 4-parameter curve fit.

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 what the optimal dilution factor is.)

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)	CORRECTED (450NM)
Blank	0 (0.123)
39.063 (optional)	0.057
78.125	0.128
156.25	0.212
312.5	0.368
625	0.673
1250	1.021
2500	1.450
5000	2.118

SPECIFICITY

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 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. This means that rat serum samples cross-react with mouse CTRP9 ELISA kit.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add **50 µl** of standard dilutions, the pretreated samples, or 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 at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 3 min.