CTRP7/C1QTNF7 (MOUSE, RAT) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE AND RAT CTRP7/C1QTNF7 CONCENTRATIONS IN EDTA PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**CTRP7/C1QTNF7 (MOUSE, RAT) ELISA KIT**
Catalog No.	SK00396-03
Formulation	96 T
Lot No.	20114810
Standard range	0.625- 40 ng/ml
Sensitivity	100 pg/ml
Sample Volume	100 µl
Dilution Factor	**8 ~ 16 (Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	EDTA Plasma
Specificity	Mouse, Rat, Human
Calibration	Human CTRP7 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8° C for 6 months, check page 2-3 for detail
This kit contains sufficient materials to run 35 - 40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This CTRP7/C1QTNF7 (Mouse, Rat) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural CTRP7 from mouse and rat plasma in a sandwich ELISA format.

This immunoassay contains recombinant CTRP7 and monoclonal antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural CTRP7 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for CTRP7. The capture antibody can bind to the CTRP7 in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against CTRP7 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, rat CTRP7 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. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_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
CTRP7 Microplate – 96 well microplate coated with an antibody specific for mouse and rat CTRP7.	**396-03-01**	**1 plate**
CTRP7 Standard – 640 ng/vial of lyophilized recombinant CTRP7.	**396-03-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrate of lyophilized biotinylated antibody against mouse, rat and CTRP7.	**396-03-03**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered solution with preservative.	**DB05**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered solution with preservative.	**DB11CI**	**1bottle**
HRP Diluent Solution – 12 mL of buffered solution with preservative.	**DB08B**	**1 bottle**
Wash Buffer – 25 mL of 20-fold concentrated buffered surfactant with preservative.	**WB01**	**1 bottle**
Substrate Solution – 11 mL of substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.25M 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 6 months. For longer storage for up to 10 months, unopened Standard, Detection Antibody Concentrate, Dilution buffer (**DB05**) 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 for 10 months.
Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

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

Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store 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

Mouse or Rat EDTA Plasma samples may require a 8-fold or 16-fold dilution. A suggested 8-fold dilution is 30 µL sample + 210 µL Dilution Buffer DB05. A suggested 16- fold dilution is 50µL per well of 8-fold diluted sample solution + 50 µL per well of Dilution Buffer DB05.

Mouse or rat Serum samples may not be detected by this ELISA Kit.

Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.

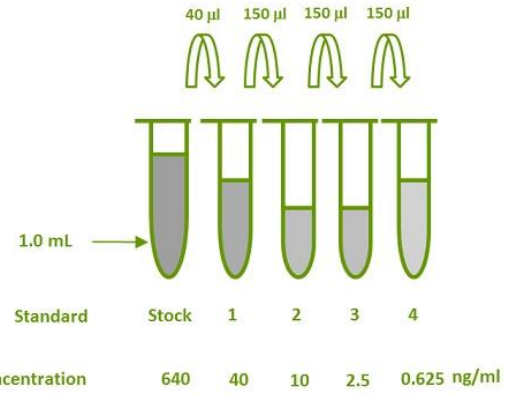
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer – If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved. Dilute 25 mL of Wash Buffer Concentrate **20X** into **475 mL** distilled or deionized water to make 500 mL of 1x Wash Buffer.

CTRP7 Standard - Reconstitute the CTRP7 standard with 1 mL of **Dilution Buffer (DB05)**. This reconstitution produces a stock solution of 640 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 450 µL of Dilution Buffer into tubes #2 to #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **40 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 ng/mL). Store the standard stock solution at -20 ~ - 70 °C for a few days.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 mL	640 ng/mL
optional	150 µL of stock	450µL	160 ng/mL
# 1	40µL of 1	600µL	40 ng/mL
# 2	150µL of 2	450µL	10 ng/mL
# 3	150µL of 3	450µL	2.5 ng/mL
# 4	150µL of 4	450µL	0.625 ng/mL



Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Antibody Diluent Solution (DB11CI)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Antibody Diluent Solution (DB11CI) into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution for 96 well test. *Freshly prepare 900 µL per 8 well strip of 1x working solution for a partial strip test. Store the 10-fold concentrated stock solution at -20 ~ -70° C for a few days.*

Streptavidin-HRP Conjugate - Pipette 10.89 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 110 µL of 100-fold concentrated stock solution to prepare working solution for 96 well test. **Note:** 1x working solution of Streptavidin-HRP should be used within 10-20 min. **(protect from light)**. **DO NOT FREEZE.** *Freshly prepare 900 µL per 8 well strip of 1x working solution of streptavidin-HRP for a partial strip test. Store the 100-fold concentrated stock solution at 2 ~ 8 °C for a few days. Always, store the 100-fold concentrated the stock solution of* Streptavidin-HRP at 2 ~ 8 °C for 10 months.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

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 (DB01)** to Blank wells.
4. Add 100 µL of **Standard dilutions from #4-1** or **samples** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
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 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 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 20 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 2 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log or 4-parameter curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

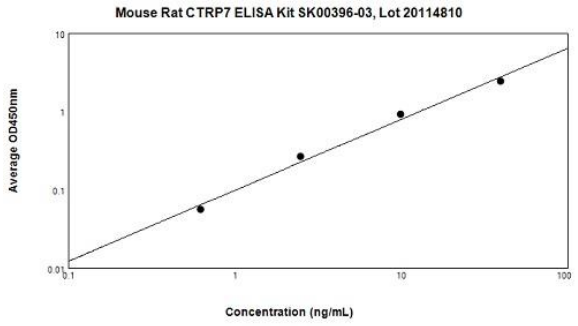
TYPICAL STANDARD VURVE

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

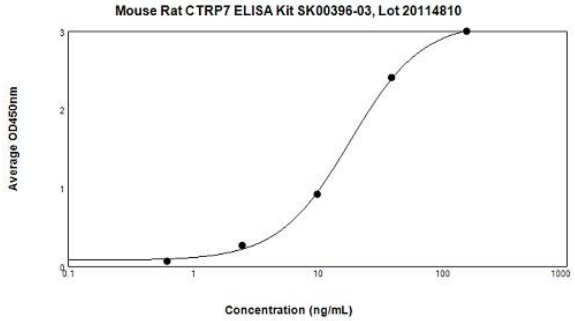
STANDARD (NG/ML)	AVERAGE OD450 NM (CORRECTED)
Blank	0 (0.073)
0.625	0.055
2.5	0.263
10	0.915
40	2.406
160 (optional)	3.000

- Lot No.: 20114810

STANDARD CURVE BY LOG-LOG FIT (0.625 ~ 40 NG/ML):



STANDARD CURVE BY 4-PARAMETER FIT (0.625 ~ 160 NG/ML):



SPECIFICITY

Proteins	Cross-reactivity
Human CTRP7	100%
Mouse CTRP15	0
Mouse CTRP6	0

Mouse or Rat EDTA plasma samples and its diluted samples can be detected this ELISA Kit. The Mouse Serum samples can not be detected by this kit.

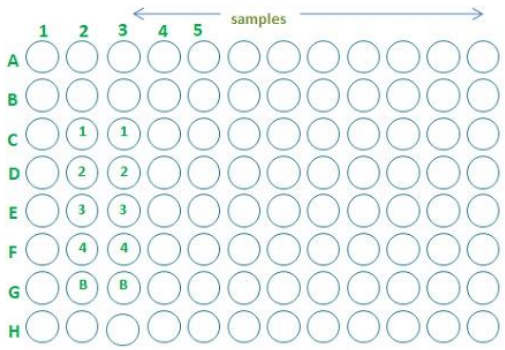
LINEARITY

To assess the linearity of the assay, pooled research Mouse or Rat EDTA Plasma samples were diluted with Dilution Buffer (DB05) and assayed.

SAMPLE TYPE	DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
Mouse EDTA Plasma	8 X	3.279	26.235	100
Mouse EDTA Plasma	16 X	1.659	26.546	101
Rat EDTA Plasma	8 X	4.545	36.359	100
Rat EDTA Plasma	16 X	2.109	33.744	93

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of standard dilutions or samples to the 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 Conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 17-22 min on plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read 450nm within 2 min.



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Expire Date: 30 July, 2024