HUMAN CTRP3 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN CTRP3 CONCENTRATIONS IN SERUM AND EDTA PLASMA.

PURCHASE INFORMATION:

ELISA Name	Human CTRP3 ELISA
Catalog No.	
Formulation	96 T
Standard range	8-5000ng/mL
Sensitivity	20 ng/mL
Sample Volume	50 µl
Dilution Factor	2
Sample Type	Serum, EDTA plasma
Specificity	Human CTRP3 only
Intra-assay Precision	4%
Inter-assay Precision	8%
Storage	4 °C

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

INTRODUCTION

Human CTRP3 ELISA employs the quantitatively competitive enzyme immunoassay technique in which human CTRP3 present in samples competed with a fixed amount of biotinylated human CTRP3 for sites on purified rabbit IgG specific against human CTRP3. During the incubation, the rabbit IgG becomes bound to the goat anti-rabbit IgG pre-coated onto the microplates. Following a wash to remove any unbound antibody, standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of human CTRP3 bound in the initial step. The sample values are then read off the standard curve.

Human CTRP3 ELSA has been shown to accurately quantitate the recombinant and natural human CTRP3. Results obtained using natural human CTRP3 showed dose response curves that were parallel to the standard curves obtained using the kit standards.

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.
_Some vials contain small quantities of material, therefore centrifuge before use.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted sulfuric 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. See material safety data sheet(s) for specific advice.

MATERIALS PROVIDED

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 2 months. For longer storage, unopened Standard, Positive Control and Biotin Solution Concentrated should be stored at -20 or -70 °C for up to 4 months. Do not use past kit expiration date.
Opened / Reconstituted Reagents: *Reconstituted Standard (5000 ng/ml), Biotin Solution and Antibody SHOULD BE STORED at -20 °C or – 70°C for up to one months. Reconstituted Biotin Solution (350 µl) CAN NOT BE STORED at 2-8°C.* Streptavidin - HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 4-6 months.
Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 4 months 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 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.

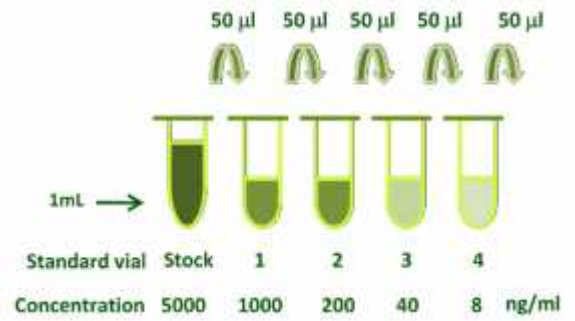
SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL sample + 100 µL Dilution Buffer. **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 1000 mL of Wash Buffer.
CTRP3 Standard - Refer to vial label for reconstitution volume. Reconstitute the **CTRP3** Standard with 1 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 200 µL of the appropriate Dilution Buffer into the tube #1 to #4. 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.

Standard	Standard	Reagent Diluent	Concentration
stock	powder	1 ml	5000 ng/ml
# 1	50µl of stock	200µl	1000 ng/ml
# 2	50µl of 1	200µl	200 ng/ml
# 3	50µl of 2	200µl	40 ng/ml
# 4	50µl of 3	200µl	8 ng/ml



Antibody- Reconstitute the **Antibody concentrated** with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 3.15 mL of **Dilution Buffer** to prepare 1 x Antibody Solution

Biotin Solution. Reconstitute the **Biotin Solution Concentrated** with 350 µl of **Dilution Buffer** to make 10-fold concentrated solution. Transfer it to 3.15 mL of Dilution Buffer to prepare 1 x Biotin Solution.

Streptavidin-HRP Conjugate - Pipette 11. 88 mL of Dilution Buffer into the 15 ml centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution. *Note: 1 x working solution of Streptavidin-HRP Conjugate should be used within a few days.*

ASSAY PROCEDURE

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

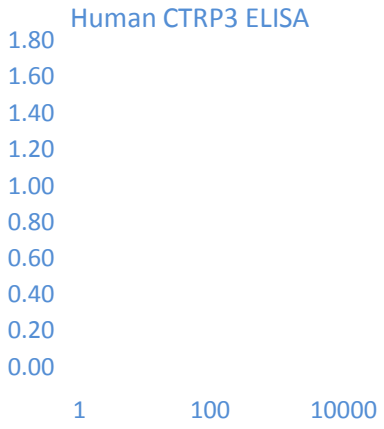
1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic bag containing the desiccant pack, reseal.
3. Leave well H1 and H2 as Blank. ***DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELL.***
4. Set A1 and A2 as total binding. Add 50 ul per well of Dilution Buffer.
5. Add 50 µl per well of standard solution from #4 to S (reverse order of serial dilution) to the appropriate wells (B1 to F2). Add 50 µl per well of Positive Control into well G1 and G2. Add 50 µl per well of samples into appropriate wells.
6. Add 25µl per well of 1 x Antibody Solution into total binding, standard, PC and samples wells. Cover or seal the plate and incubate on Microplate shaker (250-300rpm) at room temperature for 2 hours. ***Note: DO NOT ASPIRATE AND WASH PLATE. PROCEED IMMEDIATELY TO THE NEXT STEP.***
7. Add 25 µl per well of 1 x Biotin Solution into total binding, standard, PC and samples wells. Cover or seal the plate and incubate at room temperature for 2 hours. *Note: DO NOT ADD Biotin Solution to Blank wells.*
8. Aspirate wells and wash 5 times with 300 µl of 1 x Assay Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
9. Add 100 µL of **Streptavidin-HRP Conjugate** to each well. Incubate it on microplate shaker for one hour at room temperature.
10. Aspirate and wash as step 8.
11. Add 100 µL of Substrate Solution to each well. Incubate for 10-15 minutes at room temperature. **Protect from light**.
12. 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. It is recommended to add the stop solution when the total Binding or the lowest standard has developed a dark blue color.
13. Determine the optical density of each well within 30 minutes if the microplate are stored at 2 - 8°C in the dark. Using a micro-plate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate. Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, PC , and samples and subtract the average Blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

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



CALIBRATION

This immunoassay is calibrated against a highly purified E. Coli-expressed recombinant Human CTRP3 globular form.

SENSITIVITY

20 ng/mL.

RECOVERY

The recovery of CTRP3 spiked to charcoal stripped, delipidized human serum samples levels throughout the linear range of the assay was evaluated.

Spiked Conc. (ng/ml)	Conc. (ng/ml)	Recovery
0	202	
282	422	78%
370	528	88%
454	733	116%
833	1476	142%

SPECIFICITY

Human CTRP3 ELISA recognizes recombinant and natural human CTRP3. Our study data indicated that rat and mouse serum or EDTA plasma samples can be tested by this assay kit due to its samples dilution linear curves that were parallel to the standard curves.

REFERENCES

1: Maeda T, Wakisaka S. CTRP3/cartducin is induced by transforming growth factor-beta1 and promotes vascular smooth muscle cell proliferation. Cell Biol Int. 2010 Jan 29;34(3):261-6.

2: Kopp A,et al. Effects of the new adiponectin paralogous protein CTRP-3 and of LPS on cytokine release from monocytes of patients with type 2 diabetes mellitus. Cytokine. 2010 Jan;49(1):51-7. Epub 2009 Dec 1.

3: Akiyama H,et al. Elevated expression of CTRP3/cartducin contributes to promotion of osteosarcoma cell proliferation. Oncol Rep. 2009 Jun;21(6):1477-81.