HUMAN CTRP1 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN CTRP1 CONCENTRATIONS IN SERUM AND PLASMA

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN CTRP1 ELISA KIT
Catalog No.	SK00083-01
Lot No.	
Formulation	96 T
Standard Range	625-40000 pg/mL
Sensitivity	30 pg/mL
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma
Dilution factor	2-4 for serum samples (Optimal dilutions should be determined by each laboratory for each application)
Specificity	Human CTRP1 only
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2 °C-8 °C

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INTRODUCTION

Human CTRP1 immunoassay is a solid phase ELISA designed to measure human CTRP1 in serum and plasma. It contains recombinant human CTRP1 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human CTRP1. Results obtained with naturally occurring CTRP1 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 natural human CTRP1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An monoclonal antibody specific for CTRP1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CTRP1 present is bound by the immobilized antibody. After washing away any unbound substances, an antibody specific for CTRP1 is added to the wells. Following a wash to remove any unbound antibody reagent, Anti Rabbit IgG HRP conjugate is added to the wells. After washing away any unbound enzyme, Chemiluminescent reagent is added for signal development. The chemiluminescent signal [relative light units (RLU)] is measured by a chemiluminescent microplate reader. The relative light units (RLU) develops in proportion to the amount of CTRP1 bound in the initial step.

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 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
CTRP1 Microplate - 96 well white polystyrene microplate (12 strips of 8 wells) coated with an antibody against CTRP1.	**083-01-01**	**1 plate**
CTRP1 Standard – 80000 pg/vial of recombinant human CTRP1 in a buffered protein base with preservative; lyophilized.	**083-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrated of antibody against CTRP1 with preservative; lyophilized.	**083-01-03**	**1 vial**
Positive Control – one vial of recombinant human CTRP1; lyophilized.	**083-01-04**	**1 vial**
ARIG-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of ARIGHRP conjugate with preservative.	**ARIGHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservative.	**DB08**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
Luminol Enhancer - 5.5 mL of luminol enhancer solution.	**LE01**	**1 bottle**
Stable Peroxide - 5.5 mL of stable peroxide solution.	**SP01**	**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, unopened Standard, Positive Control, Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. ARIG-HRP Conjugate 100-fold concentrate **(protect from light)**, Luminol Enhancer and Stable Peroxide **(protect from light)** and other

components may be stored at 2 - 8° C for up to 6 months.
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.

OTHER SUPPLIES REQUIRED

- Chemiluminescent Microplate reader capable of measuring chemiluminescent signal at 425 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.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. 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.

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

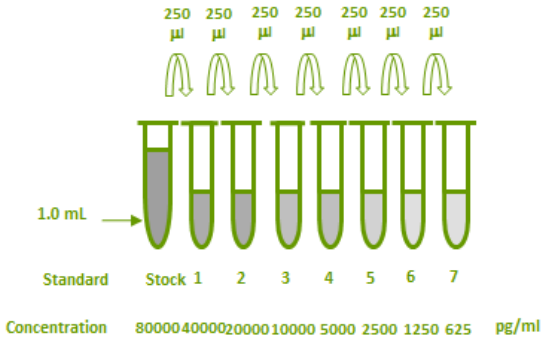
Serum samples may require 2-4 fold dilution.

Note: pooled human serum and plasma samples with pretreatment or without pretreatment were tested. The results indicate that both samples could be detectable.

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.
CTRP1 Standard - Refer to vial label for reconstitution volume. Reconstitute the **CTRP1** standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 80000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of 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 **40000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFER	CONCENTRATION
Stock	Powder	1000 µl	80000 pg/ml
# 1	250 µl of stock	250 µl	40000 pg/ml
# 2	250 µl of 1	250 µl	20000 pg/ml
# 3	250 µl of 2	250 µl	10000 pg/ml
# 4	250 µl of 3	250 µl	5000 pg/ml
# 5	250 µl of 4	250 µl	2500 pg/ml
# 6	250 µl of 5	250 µl	1250 pg/ml
# 7	250 µl of 6	250 µl	625 pg/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 Dilution Buffer

into a 15 mL centrifuge tube and transfer 1.05mL of 10-fold concentrated stock solution to prepare working solution.

ARIG-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer 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 ARIGHRP should be used within a few days **(protect from light)**.

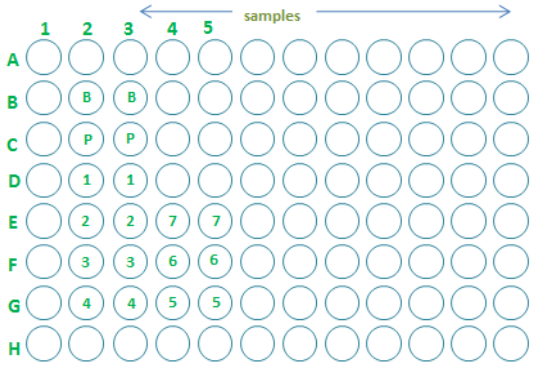
Positive Control - Reconstitute the positive control with 1 mL of Dilution Buffer to make positive control solution. **Note:** Positive Control should be used immediately.

Chemiluminescent Substrate Working Solution - mix 5.5 mL **Luminol Enhancer** with 5.5mL **Stable Peroxide** in an amber bottle. This working solution is stable for approximately 8 hours at room temperature. Exposure to the sun or any other intense light can harm the working solution. For the best result keep the working solution in an amber bottle and avoid prolonged exposure to any intense 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. 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 and 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 (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 ARIG-HRP Conjugate working solution to each well. Incubate for 1 hour on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Chemiluminescent Substrate Working Solution to each well. Incubate for 4-6 minutes at room temperature on microplate shaker. **Protect from light.**
11. Determine the chemiluminescent signal RLU [relative light units] of each well immediately, using a Chemiluminescent reader set to 425nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard RLU. 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 CTRP1 concentrations versus the log of the RLU 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.

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE RLU (CORRECTED)
625	131867
1250	189938
2500	382372
5000	623684
10000	1176342
20000	1968046
40000	2700354

*The Chemiluminescent signal RLU (relative light units) were mesured with Molecular Device FilterMax F5 Microplate Reader with integration time setting 400ms.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human CTRP1.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of CTRP1 was 30 pg/mL.

SPECIFICITY

PROTEIN	CROSSREACTIVITY (%)
Human CTRP1	**100**
Human CTRP3	**0**
Human CTRP9	**0**
Human Adiponectin	**0**
Human gAdiponectin	**0**
Human TNF-alpha	**0**
Mouse Adiponectin	**0**

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl 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 Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl ARIG-HRP conjugate working solution to each well. Incubate 1 hour on a plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl **Chemiluminescent Substrate working Solution** to each well. Incubate 4-6 min on plate shaker at RT. **Protect from light.**
↓
Read chemiluminescent signal RLU by chemiluminescent microplate reader at 425nm immediately.