

## HUMAN CTRP9 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION  
OF HUMAN CTRP9 CONCENTRATIONS IN  
EDTA PLASMA AND SERUM



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  | HUMAN CTRP9 ELISA   |
| Catalog No.   | SK00081-02  |
| Lot No.   |   |
| Formulation   | 96 T  |
| Standard range  | 3.9 - 1000 ng/mL  |
| Sensitivity   | 1 ng/mL   |
| Sample Volume   | 100 $\mu$ L   |
| Pretreatment  | Optional  |
| Sample Type   | EDTA Plasma, Serum  |
| Dilution Factor   | <b>Optimal dilutions should be determined by each laboratory for each application</b> |
| Specificity   | Human CTRP9 only  |
| Calibration   | Human CTRP9 recombinant   |
| Intra-assay Precision   | 4 - 6%  |
| Inter-assay Precision   | 8 - 12%   |
| Storage   | 2 – 8° C  |
| This kit contains sufficient materials to run 40 samples duplicated provided that assay is run according to protocol. |   |

Order Contact:  
**AVISCERA BIOSCIENCE, INC.**  
 2348 Walsh Ave., Suite C  
 Santa Clara, CA 95051  
 USA  
 Tel: (408) 982 0300  
 Fax: (408) 982 0301  
 Email: [Sales@AvisceraBioscience.com](mailto:Sales@AvisceraBioscience.com)  
[www.AvisceraBioscience.com](http://www.AvisceraBioscience.com)

**DESCRIPTION**

This Human CTRP9 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human CTRP9 from EDTA plasma and serum in a sandwich ELISA format.

This immunoassay contains recombinant human 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 human CTRP9. The capture antibody can bind to the human CTRP9 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human 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 (TMB) is added to the wells and color develops in direct proportion to the amount of human 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.

\_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        |
|--|------------------|-----------------|
| <b>CTRP9 Microplate</b> - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against CTRP9.                              | <b>081-02-01</b> | <b>1 plate</b>  |
| <b>CTRP9 Standard</b> – 1000 ng/vial of recombinant human CTRP9 in a buffered protein base with preservative; lyophilized.                       | <b>081-02-02</b> | <b>1 vial</b>   |
| <b>Detection Antibody Concentrate</b> – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against CTRP9 with preservative; lyophilized. | <b>081-02-03</b> | <b>1 vial</b>   |
| <b>Positive Control</b> - one vial of recombinant human CTRP9; lyophilized.  | <b>081-02-04</b> | <b>1 vial</b>   |
| <b>Streptavidin-HRP Conjugate</b> - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.                                | <b>SAHRP</b>     | <b>1 vial</b>   |
| <b>Dilution Buffer</b> – 60 mL of buffered protein based solution with preservative.   | <b>DB06</b>      | <b>1 bottle</b> |
| <b>Sample Buffer</b> – 20 mL of 0.1% SDS solution in PBS.  | <b>STB01</b>     | <b>1 bottle</b> |
| <b>Antibody &amp; HRP Diluent Solution</b> – 30 mL of buffered protein based solution with preservative.   | <b>DB40</b>      | <b>1 bottle</b> |
| <b>Wash Buffer</b> - 50 mL of 10-fold concentrated buffered surfactant, with preservative.   | <b>WB01</b>      | <b>1 bottle</b> |
| <b>TMB Substrate Solution</b> - 11 mL of TMB substrate solution.   | <b>TMB01</b>     | <b>1 bottle</b> |
| <b>Stop Solution</b> - 11 mL of 0.5M HCl.  | <b>S-STOP</b>    | <b>1 bottle</b> |
| <b>Plate Sealer</b>  | <b>EAPS</b>      | <b>1</b>        |
| <b>Plastic Pouch</b>   | <b>P01</b>       | <b>1</b>        |

**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.

**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.
- **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.

**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**

*Samples may be directly assayed, but if sample reading are below the detectable limit, then pretreatment may be needed before being added to the microplate. Standard and Positive Control **DO NOT NEED** to be treated.*

1. Add 240 µl of 500 mM TCEP to 11.76 mL of Sample Buffer (**STB01**) to prepare the Pretreatment Solution (10mM TCEP, 0.1% SDS in PBS, pH 7.4) (12 mL for 50 samples pretreatment).
2. Add 70 µl of sample to 210 µl of Pretreatment Solution in a polypropylene tube or vial. **Note: This pretreatment dilution (4-fold dilution) may require optimization.**

3. Vortex gently and incubate for 30 minutes at room temperature. Assay immediately and discard any excess pretreated samples.

**Optimal dilutions should be determined by each laboratory for each application. Use polypropylene test tubes.**

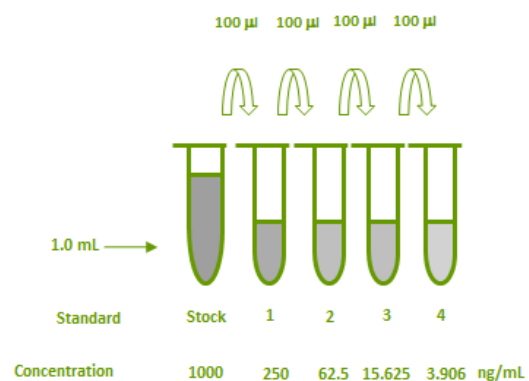
**REAGENT PREPARATION**

**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.

**Human CTRP9 Standard** - Reconstitute the CTRP9 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 1000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 300 µL of Dilution Buffer into tubes #1 to #4. Use the stock solution to produce a 4-fold dilution series (below). Mix each tube thoroughly before the next transfer. The **1000 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         | 1000 ng/ml    |
| # 1   | 100 µl of stock | 300 µl          | 250 ng/ml     |
| # 2   | 100 µl of 1     | 300 µl          | 62.5 ng/ml    |
| # 3   | 100 µl of 2     | 300 µl          | 15.625 ng/ml  |
| # 4   | 100 µl of 3     | 300 µl          | 3.906 ng/ml   |



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

**Detection Antibody Concentrate** - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody & HRP Diluent Solution (DB40)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Antibody & 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 & HRP Diluent Solution (DB40)** into a 15 mL centrifuge tube and transfer 120  $\mu$ L of 100-fold concentrated stock solution to prepare working solution (**protect from light**).

## 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. Add 100  $\mu$ L of Dilution Buffer to Blank wells.
3. Add 100  $\mu$ L of Standard dilutions in reverse order of serial dilutions, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate 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  $\mu$ L) 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  $\mu$ L of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100  $\mu$ L of Streptavidin-HRP Conjugate working solution to each well. Incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.

9. Add 100  $\mu$ L of Substrate Solution to each well. Incubate for 8-20 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100  $\mu$ L 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 within 15 minutes, using a microplate reader set to 450nm.

## 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 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.

## SPECIFICITY









| PROTEIN                   | CROSS-REACTIVITY (%) |
|---------------------------|----------------------|
| Human CTRP9 full          | 100                  |
| Human CTRP9 globular form | 100                  |
| Human CTRP3               | 0                    |
| Human CTRP1               | 0                    |
| Human CTRP6               | 0                    |
| Human Adiponectin         | 0                    |
| Human ADRP                | 0                    |

## 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 (0.117)         |
| 3.906            | 0.023             |
| 15.625           | 0.064             |
| 62.5             | 0.259             |
| 250              | 0.740             |
| 1000             | 1.645             |

**SUMMARY OF ASSAY PROCEDURE**

| <b>PREPARE REAGENTS, SAMPLES AND STANDARDS</b>   |
|--|
|   |
| Add 100 µl of standard dilutions, 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 45 minutes on the plate shaker at RT. <b>Protect from light.</b> |
|   |
| Aspirate and wash 4 times.   |
|    |
| Add 100 µl Substrate Solution to each well. Incubate 8-20 min on plate shaker at RT. <b>Protect from light.</b>                      |
|   |
| Add 100 µl Stop Solution to each well. Read 450nm within 15 min.   |