HUMAN CTRP3 ELISA KIT

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

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.

PRODUCTINFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA Name	**Human CTRP3 ELISA Kit**
Catalog No.	SK00082-03
Lot No.	
Formulation	96 T
Standard range	16 – 10000 ng/mL
Dynamic Range	16 – 10000 ng/mL
Sensitivity	3 ng/mL
Sample Volume	60 µL per well per test
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum, EDTA Plasma, Recombinant Protein
Specificity	Human, Mouse, Rat
Calibration	Human CTRP3 Globular Form Rec.
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8°C for 6 months. Check page 2 -3 for more information.
This kit contains sufficient materials to run approximately 35 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
Email: Info@AvisceraBioscience.com
sales@AvisceraBioscience.com
Website: www.AvisceraBioscience.com
www.AvisceraBioscience.net

DESCRIPTION

This Human CTRP3 ELISA kit contains the necessary components required for the quantitative measurement of natural human CTRP3 from serum and plasma in a competitive EIA format.

This immunoassay contains human CTRP3 pre-coated microplates and human CTRP3 Globular Form standard and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify natural CTRP3.

ASSAY OVERVIEW

The Human CTRP3 ELISA Kit employs the quantitatively competitive enzyme immunoassay technique in which CTRP3 present in samples or CTRP3 Protein Standards were pre-incubated with Anti Human CTRP3 antibody, then pre-incubated antibody solutions compete with a fixed amount of CTRP3 proteins on the pre-coated microplate. Following a wash to remove any unbound primary antibody, standard and samples, the High Sensitivity Goat Anti Rabbit IgG HRP Conjugate was added to each well of Elastin Microplate. Following a final wash to remove any unbound enzyme, a TMB substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when stop solution is added. The intensity of the color measured is in inverse proportion to the amount of elastin immunoreactivity bound in the initial step. The higher the concentration of the CTRP3 protein in solution is, the less the antibody bound to the plate will be. 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.

_ 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.

_ 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.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
CTRP3-Microplate - 96 well microplate pre-coated with Human CTRP3 Globular Form Recombinant.	**082-03-01**	**1 plate**
CTRP3 Standard – 10000 ng/vial of human CTRP3 GF Rec. in a buffered protein base with preservative; lyophilized.	**082-03-02**	**1 vial**
Antibody Solution Concentrate – 1 mL /vial, 10-fold concentrate of polyclonal purified IgG against human CTRP3 GF with preservative; lyophilized.	**082-03-03**	**1 vial**
Anti Rabbit IgG (H+L) HRP Conjugate - 10 µl/vial, 5000-fold concentrated solution of Anti Rabbit IgG conjugate to HRP with preservative.	**ARIGHRP**	**1 vial**
Dilution Buffer – 50 mL of buffered protein based solution.	**DB01**	**1 bottle**
HRP Diluent Solution – 30 mL of buffered protein based solution with preservative.	**DB08C**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 Bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution	**TMB01**	**1 Bottle**
Stop Solution - 11 mL of 0.25M HCl.	**S-STOP**	**1 Bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 8 months. For longer storage up to 10 months, unopened Standard, Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20°C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2 - 8°C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of measuring absorbance at 450 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.
- 1.5 ml or 0.5 ml of microcentrifuge vails
- 200 µL of 96 well PCR plate or 8 well PCR strip.

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.

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

SAMPLE PREPARATION

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.

Preparation of Dilution Buffer

Elastin Standard - Reconstitute the CTRP3 standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 10000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 µL of Dilution Buffer into tubes #2 to #6. Use the stock solution to produce a 5-fold dilution series (see below). Mix each tube thoroughly before the next transfer. The **10000 ng/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard name as Total Binding (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	10000 ng/ml
# 1	100 µl of stock	400 µl	2000 ng/ml
# 2	100 µl of 1	400 µl	400 ng/ml
# 3	100 µl of 2	400 µl	80 ng/ml
# 4	100 µl of 3	400 µl	16 ng/ml
Total Binding		500 µl	0

Antibody Solution - Reconstitute the Antibody Solution Concentrate with 1000 µL of **Dilution Buffer** to produce a 10-fold concentrated stock solution. Transfer 0.7 of 10-fold concentrated stock solution to 6.3 mL of **Dilution Buffer** to prepare 1x Antibody Solution.

Anti Rabbit IgG-HRP Conjugate - Transfer 2 µL of 5000-fold concentrated stock solution to 10 mL of **HRP Diluent Solution (DB08C)** to prepare working solution. **Protect from light. Note:** 1 x working solution of Anti rabbit IgG HRP should be freshly prepared and used within a few hours.

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. Pre-incubation 60 µL of standard, total binding (zero standard) and sample solutions with 60 µL of 1 x detection antibody in the wells of PCR plate (96 wells) (or 1.5 ml of microcentrifuge vials) for 1 hour on Microplate shaker (300 rpm). Set 12 wells for standard, 2 wells for total binding. Set 38 or more wells for sample assay. 2.1. Add 60 µL of 1 x working solution of detection antibody to each vial or well. 2.2. Add 60 µL of each standard solution and 60 µL of sample solution into sample assay vials. Add 60 µL of Dilution Buffer in total binding serves as the zero standard (0 ng/mL).
   Cover the PCR plate with plate sealer and incubate on microplate shaker (200rpm) at room temperature for 2 hours.

3. After two hours for pre-incubation, transfer 100 µL of above pre-incubated solutions into each well of CTRP3 Microplate (082-03-01). Cover with plate sealer and incubate on microplate shaker (300rpm) at room temperature for 1 hours.
4. Leave two wells as Blank. ***DO NOT ADD ANY ANTIBODY OR SAMPLES INTO BLANK WELLS.***
5. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µL of Anti Rabbit IgG -HRP Conjugate working solution to each well, including blanks. Incubate on microplate shaker for 45 minutes at room temperature. **Protect from light.**
7. Aspirate and wash as step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate on microplate shaker for 15 minutes at room temperature. **Protect from light**.
9. 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.
10. Determine the optical density of each well using a microplate reader set to 450 nm within 5 min.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, and sample, 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

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

Well	Average OD450nm	Standard (ng/mL)
Total Binding	1.791	0
Standard 4	1.632	16
Standard 3	1.423	80
Standard 2	0.726	400
Standard 1	0.212	2000
Stock	0.144	10000

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human CTRP3 Globular Form Rec.	100
Mouse CTRP3 Globular Form Rec	100
Human CTRP9 Globular Form Rec.	0
Human CTRP1 Globular Form Rec.	0

The mouse and rat serum samples can be tested by this Human CTRP3 ELISA Kit

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⇩
Add 60 µl of standard dilutions, samples to the well. Add 60 µL of 1x Antibody solution to each pre-incubation vial or PCR well, except blanks. **Incubate 2 hours** on the microplate shaker at RT. **DO NOT WASH OR ASPIRATE. PROCEED TO NEXT STEP.**
⇩
Transfer 100 µL of above pre-incubated Solution to each well of CTRP3 Plate (082-03-01) use a 8 channel Pipettes, except blanks. **Incubate 1 hour** on the plate shaker at RT.
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Anti Rabbit IgG-HRP conjugate working solution to all wells, including blanks. Incubate 45 minutes on the plate shaker at RT. **Protect from light.**
⇩
Aspirate and wash 4 times.
⇩
Add 100 µl Substrate Solution to each well. Incubate 15 min on plate shaker at RT. **Protect from light.**
⇩
Add 100 µl Stop Solution to each well. Read at 450nm within 5 min.