HUMAN VASOSTATIN-2 / CHROMOGRANIN A (19-131) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF VASOSTATIN-2 CONCENTRATIONS IN HUMAN 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.

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HUMAN VASOSTATIN-2/CHROMOGRANIN A (19-131) ELISA**
Catalog No.	SK00084-01
Lot No.	
Formulation	96 T
Standard range	0.64 - 2000 ng/mL
Dynamic range	1 - 100 ng/mL
Sensitivity	0.32 ng/mL
Sample Volume	50 µl per well
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum, EDTA Plasma
Specificity	Human Vasostatin-2
Calibration	Human Vasostatin-2 recombinant
Intra-assay Precision	6 – 8%
Inter-assay Precision	12 – 14%
Storage	2 – 8° C
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
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

DESCRIPTION

Vasostatin-2/Chromogranin A (19-131) (Human) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Vasostatin-2 from serum and plasma in a competitive enzyme immunoassay technique format.

This immunoassay contains recombinant Vasostatin-2 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural human Vasostatin-2 samples.

ASSAY OVERVIEW

Human Vasostatin-2 ELISA employs the quantitatively competitive enzyme immunoassay technique in which Vasostatin-2 present in samples compete with a fixed amount of biotinylated Vasostatin-2 for sites on an antibody specific against Vasostatin-2. Following a wash to remove any unbound standard, positive control, samples, antibody 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 Stop Solution is added. The intensity of the color measured is in inverse proportion to the amount of human Vasostatin-2 bound in the initial step. The sample values are then 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.
_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
R-Microplate - 96 well microplate pre-coated with polyclonal anti rabbit IgG Fc purified IgG.	**RM01**	**1 plate**
Vasostatin-2 Standard – refer to lot specific of recombinant human Vasostatin-2 in a buffered protein base with preservative; lyophilized.	**084-01-01**	**1 vial**
Biotin Solution Concentrate – refer to lot specific of 10-fold concentrate of human Vasostatin-2 biotinylated with preservative; lyophilized.	**084-01-02**	**1 vial**
Vasostatin-2 Antibody Concentrate – refer to lot specific of 10-fold concentrate of polyclonal purified IgG against human Vasostatin-2 with preservative; lyophilized.	**084-01-03**	**1 vial**
Positive Control – one vial of recombinant human Vasostatin-2; lyophilized (optional).	**084-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	**DB08B**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB68C**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M 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 8 months. For longer storage, unopened Standard, Positive Control, Antibody Concentrate and Biotin Solution 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.

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

Serum and plasma samples DO NOT need to be diluted. **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.

Vasostatin-2 Standard - Reconstitute the Vasostatin-2 standard with refer to lot specific of Dilution Buffer. Pipette 200 µL of Dilution Buffer into tubes #2 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 ng/mL** standard serves as the high standard.

Tube	Standard	Dilution Buffer	Concentration
Stock	powder	Refer to lot specific	
# 1	Refer to lot specific	Refer to lot specific	2000 ng/ml
# 2	50µl of 1	200µl	400 ng/ml
# 3	50µl of 2	200µl	80 ng/ml
# 4	50µl of 3	200µl	16 ng/ml
# 5	50µl of 4	200µl	3.2 ng/ml
# 6	50µl of 5	200µl	0.64 ng/ml

Positive Control - Reconstitute the Positive Control with refer to lot specific of Dilution Buffer.

Vasostatin-2 Antibody Concentrate - Reconstitute the Vasostatin-2 Antibody Concentrate with refer to lot specific of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer 0.35ml of 10-fold stock solution to 3.15 mL of Dilution Buffer to prepare **1x Antibody Solution**.

Biotin Solution Concentrate - Reconstitute the Biotin Solution Concentrate with refer to lot specific of Dilution Buffer to make 10-fold concentrated stock solution. Transfer 0.35ml of 10-fold stock solution to 3.15 mL of Dilution Buffer to prepare **1x Biotin Solution**.

Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 11.88 mL of **HRP Diluent Solution (DB68C)** 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. Leave two wells as Blank. ***DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.***
3. Set two wells as Total Binding. Add 50 µl per well of **Dilution Buffer**.
4. Add 50 µL per well of **Standard dilutions** from #5 to #S (reverse order of serial dilution) to the appropriate wells. Add 50 µL per well of **Positive Control** into another wells. Add 50 µL per well of **samples** into other wells.
5. Add 25 µL per well of **1x Antibody Solution** into total binding, standard, positive control and sample wells. Cover with plate sealer 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.***
6. Add 25 µL per well of **1x Biotin Solution** into total binding, standard, positive control and sample wells. Cover with plate sealer and incubate on microplate shaker at room temperature for 2 hours. **Note:** DO NOT ADD Biotin Solution to Blank wells.
7. Aspirate wells and wash 4 times with 300 µl of **1x Wash Buffer**. Blot plate on absorbent paper to remove any residual buffer.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well, including the blanks. Incubate on microplate shaker for one hour at room temperature. **Protect from light**.
9. Aspirate and wash as step 7.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for refer to lot specific 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. It is recommended to add the stop solution when the total Binding or the lowest standard has developed a dark blue color.
12. Determine the optical density of each well using a microplate reader set at 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control 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

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

Well	OD450 reading	Standard (ng/mL)
Blank	Refer to lot	
Total Binding	Refer to lot	0
Standard 5	2.093	0.64
Standard 4	1.678	3.2
Standard 3	0.942	16
Standard 2	0.464	80
Standard 1	0.173	400
Standard S	0.064	2000

SPECIFICITY

Proteins	Cross-reactivity
Human Vasostatin-2	100%
Rat Vasostatin-2	100%
Human Periostin	0
Human EGF	0
Human sHB-EGF	0
Human VEGF	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 50 µL of standard dilutions, samples, or positive control to the wells. Add 25 µL of 1x Antibody solution to each well. Incubate 2 hours on the plate shaker at RT. **Do not wash or aspirate. Proceed to next step.**
↓
Add 25 µL 1x Biotin 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 all wells, including blanks. Incubate 1 hour on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate refer to lot specific on the plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read at 450nm.