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

FOR THE QUANTITATIVE DETERMINATION OF HUMAN VASOSTATIN-2/CHGA (19-131) CONCENTRATIONS IN SERUM AND EDTA PLASMA

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN VASOSTATIN-2/CHGA (19-131) ELISA
Catalog No.	SK00084-02
Lot No.:	20110873
Formulation	96 T
Standard range	78-5000 pg/ml
Sensitivity	10 pg/mL
Sample Volume	100 µl
Dilution Factor	*Optimal dilutions should be determined by each laboratory for each application*
Sample Type	Serum, EDTA Plasma
Specificity	Human Vasostatin-2
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2-8 °C

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INTRODUCTION

Human Vasostatin-2 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human Vasostatin-2 in serum and EDTA plasma. It contains recombinant human Vasostatin-2 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human Vasostatin-2. Results obtained with naturally occurring Vasostatin-2 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 Vasostatin-2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Vasostatin-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Vasostatin-2 present is bound by the immobilized antibody. After washing away any unbound substances, an antibody specific for Vasostatin-2 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, a substrate solution is added to the wells and color develops in proportion to the amount of vasostatin-2 bound in the initial step. The color development is stopped and the intensity of the color is measured.

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 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.

_ 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
VASOSTATIN-2 Microplate – 96 well microplate precoated with anti-human Vasostatin-2 antibody	084-02-01	1 plate
VASOSTATIN-2 Standard – 10,000 pg/vial of recombinant human Vasostatin-2 in a buffered protein base with preservatives; lyophilized.	084-02-02	1 vial
VASOSTATIN-2 Antibody Concentrate – 1.05 ml/vial, 10-fold concentrate of an antibody against human Vasostatin-2 with preservatives; lyophilized.	084-02-03	1 vial
Positive Control – one vial of recombinant human Vasostatin-2 , lyophilized (optional)	084-02-04	1 vial
Anti Rabbit IgG-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	ARIGHRP	1 vial
Dilution Buffer - 60 mL of buffered protein based solution with preservatives	DB18	1 bottle
HRP Diluent Solution - 12 mL of buffered protein based solution with preservatives	DB08	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 0.5M HCl	S-STOP	1 bottle
Plate Sealer	EAPS	1
Plastic Pouch	P01	1

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20 °C or – 70°C for up to one month. ARIG-HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 12 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C after opening.

OTHER SUPPLIES 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.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric 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.

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.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

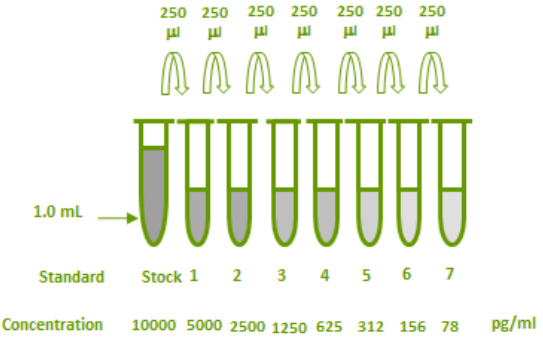
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

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 Wash Buffer.

VASOSTATIN-2 Standard - Refer to vial label for reconstitution volume. Reconstitute the **VASOSTATIN-2** Standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into the tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **5000 pg/mL** standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 pg/mL).

STANDARD TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	10000 pg/ml
# 1	250µl of stock	250µl	5000 pg/ml
# 2	250µl of 1	250µl	2500 pg/ml
# 3	250µl of 2	250µl	1250 pg/ml
# 4	250µl of 3	250µl	625 pg/ml
# 5	250µl of 4	250µl	312 pg/ml
# 6	250µl of 5	250µl	156 pg/ml
# 7	250µl of 6	250µl	78 pg/ml



VASOSTATIN-2 Antibody Concentrate - Reconstitute the Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 9.45 mL of Dilution Buffer to prepare 1x Antibody solution.

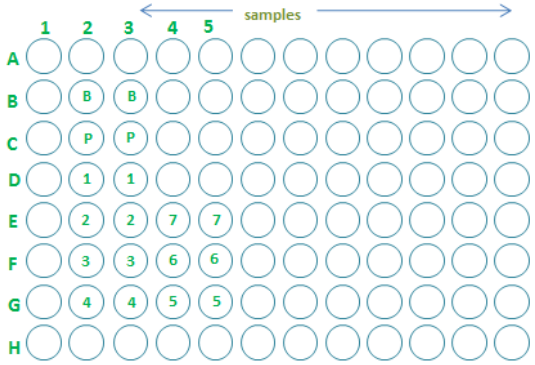
Anti Rabbit IgG-HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 11.88 ml of **HRP Diluent Solution (DB08)** to prepare working solution. **Note:** 1x working solution of Anti Rabbit IgG-HRP Conjugate should be used within a few days.

Positive Control- Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

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 duplicates.

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 pouch with the desiccant pack and seal.
3. Leave wells B2 and B3 as Blank. Add 100 µl per well of Dilution Buffer.
4. Add 100 µl per well of standard solution from #7 to #1 (reverse order of serial dilution) to the appropriate wells (D2, D3 to G2, G3 and G4, G5 to E4, E5). Add 100 µl per well of Positive control into wells C2 and C3. Add 100 µl per well of samples into appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250 rpm). Note: Standard, Blank and PC should be assayed in duplicates.
5. Aspirate wells and wash 4 times with 300 µl of 1x Assay Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µl per well of 1x Antibody solution. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Anti Rabbit IgG-HRP Conjugate working solution**. Cover or seal the plate and incubate at room temperature for 60 minutes on microplate shaker. **Protect from light**.
11. Repeat the aspiration/wash as in step 5.
12. Add 100 µL of Substrate Solution to each well. Incubate for 10-20 minutes at room temperature. **Protect from light**.
13. 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.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 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 log-log 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.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human Vasostatin-2.

SENSITIVITY

The minimum detectable dose (MDD) of human Vasostatin-2 was 10 pg/mL.

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)	OD450 READING
0 (Blank)	0 (0.136)
78	0.024
156	0.036
312	0.075
625	0.136
1250	0.263
2500	0.439
5000	0.944

Lot No.:

Positive Control: 250 ~ 550 pg/ml

SPECIFICITY

This assay recognizes both natural and recombinant human Vasostatin-2. No significant cross-reactivity or interference was observed. The data indicated that mouse serum or plasma sample does not show any cross-reactivity with this ELISA Kit.

PROTEIN	CROSSREACTIVITY (%)
Human Vasostatin-2	100
Rat Vasostatin-2	0
Human Vasostatin-1	0
Human BDNF	0
Human Periostin	0
Mouse Periostin	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 1x Antibody Solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Add 100 µl Anti Rabbit IgG HRP conjugate working solution to all wells. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 10-20 min on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min