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

FOR THE QUANTITATIVE DETERMINATION OF VASOSTATIN-2 CONCENTRATIONS IN HUMAN SERUM AND EDTA PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN VASOSTATIN-2/ CHROMOGRANIN A (19-131) ELISA
Catalog No.	SK00084-01
Lot No.	
Formulation	96 T
Standard Range	0.64-400 ng/mL
Dynamic Range	0.64-400 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
Intra-assay Precision	6 - 8%
Inter-assay Precision	12 - 14%
Storage	2 - 8°C

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

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INTRODUCTION

Human Vasostatin-2 ELISA employs the quantitatively competitive enzyme immunoassay technique in which human Vasostatin-2 present in samples compete with a fixed amount of biotinylated human Vasostatin-2 for sites on purified rabbit IgG specific against human Vasostatin-2. During the incubation, the rabbit IgG becomes bound to the goat anti-rabbit IgG pre-coated onto the microplates. Following a wash to remove any unbound antibody, standard, samples 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 the 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.

Human Vasostatin-2 ELISA has been shown to accurately quantify the recombinant and natural human Vasostatin-2. Results obtained using natural human Vasostatin-2 showed dose response curves that were parallel to the standard curves obtained using the kit standards.

LIMITATIONS OF THE PROCEDURE

_ 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.
_ If samples generate values higher than the highest standard, dilute 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.
_Some vials contain small quantities of material, therefore centrifuge before use.

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.

MATERIALS 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 – 1 μg/vial of recombinant human Vasostatin-2 in a buffered protein base with preservatives; lyophilized.	**084-01-01**	**1 vial**
Biotin Solution Concentrated - 350 μL/vial, 10-fold concentrated of human Vasostatin-2 biotinylated with preservatives; lyophilized.	**084-01-02**	**1 vial**
VASOSTATIN-2 Antibody Concentrated – 350 μL/vial, 10-fold concentrated of polyclonal purified IgG against human Vasostatin-2 with preservatives; 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**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservatives	**DB08**	**1 bottle**
Dilution Buffer – 60 mL of buffered protein based solution with preservatives. Ready to use.	**DB18**	**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 6 months. For longer storage, unopened Standard, Positive Control, Antibody Concentrated and Biotin Solution Concentrated should be stored at -20 or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard, Positive Control, Biotin Solution and Antibody Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Reconstituted Biotin Solution CAN NOT BE STORED at 2 - 8°C. Streptavidin-HRP Conjugate 100-fold concentrated 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 and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 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.

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.
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.

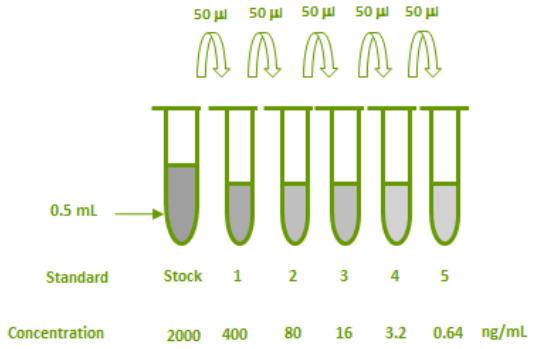
REAGENT PREPARATION

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 0.5 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 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 #1 to #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 400 ng/mL standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	0.5 ml	2000 ng/ml
# 1	50µl of stock	200µl	400 ng/ml
# 2	50µl of 1	200µl	80 ng/ml
# 3	50µl of 2	200µl	16 ng/ml
# 4	50µl of 3	200µl	3.2ng/ml
# 5	50µl of 4	200µl	0.64 ng/ml



Antibody Concentrate - Reconstitute the Antibody Concentrate with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 3.15 mL of Dilution Buffer to prepare 1x Antibody Solution.

Biotin Solution Concentrate - Reconstitute the Biotin Solution Concentrate with 350 µl of Dilution Buffer to make 10-fold concentrated solution. Transfer it to 3.15 mL of Dilution Buffer to prepare 1x Biotin Solution. **Note:** 1x working solution of Biotin SHOULD BE STORED at -20°C or -70°C.

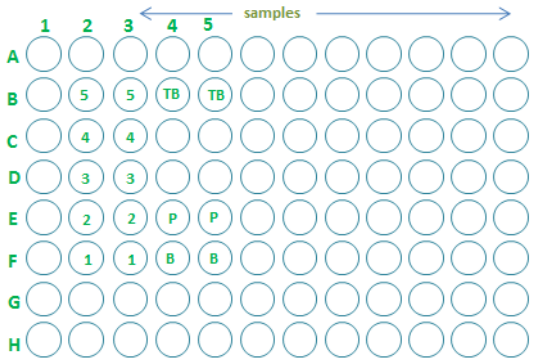
Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 11.88 mL of **HRP Diluent Solution (DB08)** to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

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 F4 and F5 as Blank. **DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.**
4. Set B4 and B5 as total binding (TB). Add 50 µl per well of Dilution Buffer.
5. Add 50 µl per well of standard solution from #5 to #1 (reverse order of serial dilution) to the appropriate wells (B2, B3 to F2, F3). Add 50 µl per well of Positive Control into wells E4 and E5. Add 50 µl per well of samples into appropriate wells.
6. Add 25 µl per well of 1x Antibody Solution into total binding, standard, positive control and sample wells. Cover with the 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.**
7. Add 25 µl per well of 1x Biotin Solution into total binding, standard, positive control and sample wells. Cover with the plate sealer and incubate at room temperature for 2 hours. **Note: DO NOT ADD Biotin Solution to Blank wells.**
8. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
9. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate it on microplate shaker for 60 minutes at room temperature. **Protect from light.**
10. Aspirate and wash as step 8.
11. Add 100 µL of Substrate Solution to each well. Incubate for 4-6 minutes at room temperature. **Protect from light**.
12. 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.
13. Determine the optical density of each well within 15 minutes using a micro-plate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate. Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the standards.



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.

Well	OD450 reading	Standard (ng/mL)
Blank	0.081	
Total Binding	0.895	0
Standard 5	0.756	0.64
Standard 4	0.604	3.2
Standard 3	0.354	16
Standard 2	0.228	80
Standard 1	0.118	400

- Lot No.:
- Positive Control: 30-70 ng/mL

CALIBRATION

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

SENSITIVITY

0.32 ng/mL

SPECIFICITY

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

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

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Add 50 µl of standard, samples, positive control to each well. Add 25 µL of 1x Antibody solution to each well. Incubate 2 hours on the plate shaker at RT. **Note: Do not aspirate or wash. Proceed immediately to the next step.**

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Add 25 µl 1x Biotin Solution to each well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 100 µl Streptavidin-HRP conjugate working solution to all wells. Incubate 60 min on the plate shaker at RT. **Protect from light.**

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Aspirate and wash 4 times.

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Add 100 µl Substrate Solution to each well. Incubate 4-6 min on the plate shaker. **Protect from light.**

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Add 100 µl Stop Solution to each well. Read 450nm within 15 min