RAT VASOSTATIN-2 / CHROMOGRANIN A (19-146) ELISA KIT

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

PURCHASE INFORMATION:

ELISA Name	Rat Vasostatin-2 / Chromogranin A (19-146) ELISA
Catalog No.	SK00085-01
Lot No.	
Formulation	96 T
Standard range	12.8-200000 pg/ml
Dynamic range	64-40000 pg/ml
Sensitivity	10-12.8 pg/ml
Sample Volume	50 µl per well
Dilution Factor	5 (**Optimal dilutions should be determined by each laboratory for each application**)
Sample Type	Serum, EDTA plasma
Specificity	Rat, Mouse
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

Rat VASOSTATIN-2 ELISA employs the quantitatively competitive enzyme immunoassay technique in which Rat VASOSTATIN-2 present in samples competed with a fixed amount of biotinylated Rat VASOSTATIN-2 for sites on purified rabbit IgG specific against Rat 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 proportion to the amount of Rat VASOSTATIN-2 bound in the initial step. The sample values are then read off the standard curve.

Rat VASOSTATIN-2 ELSA has been shown to accurately quantitate the recombinant and natural Rat VASOSTATIN-2. Results obtained using natural Rat 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 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.
_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 Rat VASOSTATIN-2 in a buffered protein base with preservatives; lyophilized	**085-01-01**	**1 vial**
Biotin Solution Concentrate - 350 µL/vial, 10-fold concentrated of Rat VASOSTATIN-2 biotinylated with preservatives; lyophilized	**085-01-02**	**1 vial**
VASOSTATIN-2 IgG Concentrate – 350 µl/vial, 10-fold concentrated of polyclonal purified IgG against Rat VASOSTATIN-2 with preservatives; lyophilized	**085-01-03**	**1 vial**
Positive Control – one vial of recombinant Rat VASOSTATIN-2 , lyophilized (optional)	**085-01-04**	**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/bottle of buffered protein based solution with preservatives	**DB06**	**1 bottle**
Dilution Buffer - 60mL/bottle of buffered protein based solution with preservatives. Ready to use.	**DB18**	**1 bottle**
Wash Buffer - 50 ml/bottle, 10-fold concentrated buffered surfactant, with preservative	**WB01**	**1 bottle**

TMB Substrate Solution - 11 ml/bottle of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution - 11 ml/bottle of contains 0.5 M 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 12 months. For longer storage, unopened Standard, Positive Control and Biotin Solution Concentrated should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard (1000 ng/ml), Biotin Solution , Positive Control and IgG concentrate SHOULD BE STORED at -20 °C or – 70°C for up to one month. Reconstituted Biotin Solution (350 µl) 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 12 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal 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, 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

Serum and plasma samples may need a 5-fold dilution. A suggested 5-fold dilution is 25 µL sample + 100 µL Dilution Buffer. ***Optimal dilutions should be determined by each laboratory for each application.***

Use polypropylene test tubes.

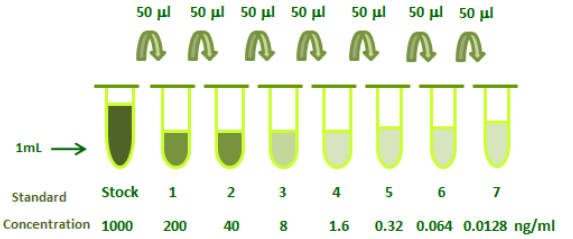
REAGENT PREPARATION

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 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 200 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 ng/mL standard serves as the high standard.

Tube	Standard	Dilution Buffer	Concentration
Stock	powder	1 ml	1000 ng/ml
# 1	50µl of stock	200µl	200 ng/ml
# 2	50µl of 1	200µl	40 ng/ml
# 3	50µl of 2	200µl	8 ng/ml
# 4	50µl of 3	200µl	1.6 ng/ml
# 5	50µl of 4	200µl	0.32 ng/ml
# 6	50µl of 5	200µl	0.064 ng/ml
# 7	50µl of 6	200µl	0.0128 ng/ml



IgG Concentrate - Reconstitute the **IgG 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 IgG Solution**.

Biotin Solution - 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**.

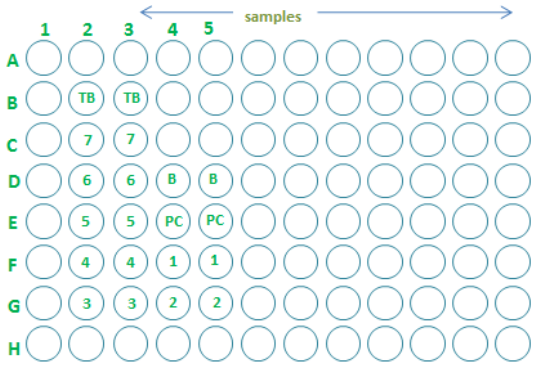
Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 11.88 mL of **HRP Diluent Solution** to prepare working solution. **Note**: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. *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, reseal.
3. Leave well D4 and D5 as Blank. ***DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.***
4. Set B2 and B3 as total binding. Add 50 µl per well of **Dilution Buffer**.
5. Add 50 µl per well of **standard solution** from #7 to #1 (reverse order of serial dilution) to the appropriate wells (C2, C3 to G2, G3, and F4, F5 to G4, G5). Add 50 µl per well of **Positive Control** into wells E4, E5. Add 50 µl per well of **samples** into appropriate wells.
6. Add 25µl per well of **1x IgG Solution** into total binding, standard, positive control and samples wells. Cover or seal the plate 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 samples wells. Cover or seal the plate 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 on microplate shaker for one hour 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 3-8 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. Set the microplate reader to 450 nm.
14. 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 (pg/mL)
Blank	0.071	
Total Binding	1.573	0
Standard 7	1.592	12.8
Standard 6	1.510	64
Standard 5	1.318	320
Standard 4	1.025	1600
Standard 3	0.549	8000
Standard 2	0.262	40000
Standard 1	0.156	200000

- *Lot No.:
- Positive Control: 0.8 – 1.8 ng/ml

CALIBRATION

This immunoassay is calibrated against a highly purified E. Coli-expressed recombinant Rat Vasostatin-2.

SENSITIVITY

10-12.8 pg/ml

SPECIFICITY

Proteins	Cross-reactivity
Rat Vasostatin-2,	100%
human Vasostatin-2	90%
Rat Visfatin	0
Rat Leptin	0
Rat FABP-4	0
Rat gAdiponectin	0
Mouse FGF-21	0

Rat Vasostatin-2 ELISA recognizes recombinant and natural Rat Vasostatin-2. The data indicated that mouse serum or EDTA plasma samples can be tested by this assay kit due to its samples dilution linear curves that were parallel to the standard curves.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⇩
Add 50µl of **standard, samples, positive control** to each well. Add 25 µL of **1X IgG 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. 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 3-8 min on the bench top. **Protect from light**.
⇩
Add 100 µl **Stop Solution** to each well. Read 450nm within 15 min