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

PRODUCT 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
Calibration	Rat Vasostatin-2 Recombinant
Intra-assay Precision	6-8%
Inter-assay Precision	12-14%
Storage	2-8°C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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.

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

DISCRIPTION

This Rat VASOSTATIN-2 ELISA kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Rat VASOSTATIN-2 from serum and plasma in a competitive EIA format.

This immunoassay contains recombinant and biotinylated recombinant Rat VASOSTATIN-2, and an antibody raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural VASOSTATIN-2.

ASSAY OVERVIEW

This assay employs the quantitative competitive EIA format. Rat VASOSTATIN-2 present in samples competes with a fixed amount of biotinylated Rat VASOSTATIN-2 for sites on an antibody specific against Rat VASOSTATIN-2. After a wash to remove any unbound 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 Stop Solution is added. The intensity of the color measured is in inverse proportion to the amount of Rat VASOSTATIN-2 bound in the initial step. 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.
_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. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_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 – 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 Antibody Concentrate – 350 µl/vial, 10-fold concentrated of polyclonal purified antibody 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 6 months. For longer storage, unopened Standard, Positive Control, Antibody Concentrate and Biotin Concentrate should be stored at -20 or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (1000 ng/mL), Biotin concentrated solution and Antibody concentrated solution 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 solution (**protect from light**) 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.

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.

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 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.

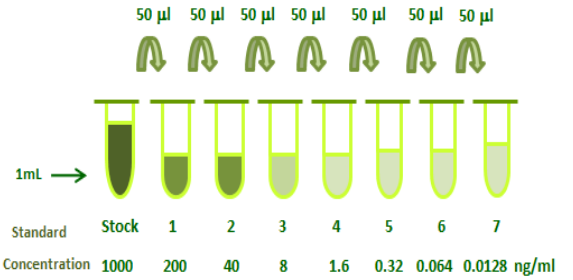
REAGENT PREPARATION

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



VASOSTATIN-2 Antibody Concentrate - Reconstitute the **VASOSTATIN-2 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 - 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 (protect from light).

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. Note: *Positive Control should be prepared and used within a few days if store at -20 or -70 °C.*

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. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Leave two wells as Blank. ***DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.***
4. Set two wells 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. Add 50 µl per well of **Positive Control** into appropriate wells. Add 50 µl per well of **samples** into appropriate wells.
6. Add 25µl per well of **1x Antibody 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 3-7 minutes on microplate shaker 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.

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 new standard curve should be generated for each set of samples assayed.

Well	OD450 reading	Standard (pg/mL)
Blank	0.099	
Total Binding	1.215	0
Standard 7	1.088	12.8
Standard 6	1.103	64
Standard 5	0.928	320
Standard 4	0.706	1600
Standard 3	0.397	8000
Standard 2	0.216	40000
Standard 1	0.103	200000

- Lot No.:
- Positive Control:
-

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 the well. 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. 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 3-7 min on the plate shaker. **Protect from light**.
⬇
Add 100 µl **Stop Solution** to each well. Read 450nm within 15 min