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

FOR THE QUANTITATIVE DETERMINATION OF RAT VASOSTATIN-2/CHGA (19-146) CONCENTRATIONS IN SERUM AND 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:

ELISA NAME	RAT VASOSTATIN-2/CHGA (19-146) ELISA
Catalog No.	SK00085-06
Lot No.:	
Formulation	96 T
Standard range	400-25600 pg/ml
Sensitivity	400 pg/mL
Sample Volume	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum and EDTA plasma
Specificity	Rat Vasostatin-2
Calibration	Rat Vasostatin-2 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

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 sandwich ELISA format.

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

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for Rat Vasostatin-2. The capture antibody can bind to the Rat Vasostatin-2 in the standard and samples. After washing the plate of any unbound substances, an antibody against Rat Vasostatin-2 is added to the wells. After another washing of the plate, Goat Anti Rabbit IgG-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of Rat Vasostatin-2 bound in the standard solutions or samples. 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
Vasostatin-2-Microplate – 96 well microplate precoated with anti-Rat Vasostatin-2 Antibody.	**085-06-01**	**1 plate**
Vasostatin-2 Standard – 25600 pg/vial of recombinant rat Vasostatin-2 in a buffered protein base with preservative; lyophilized.	**085-06-02**	**1 vial**
Vasostatin-2 Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of an antibody against rat Vasostatin-2 with preservative; lyophilized.	**085-06-03**	**1 vial**
Positive Control – one vial of recombinant rat Vasostatin-2; lyophilized (optional).	**085-06-04**	**1 vial**
Anti Rabbit IgG-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Goat Anti Rabbit IgG conjugate to HRP.	**ARIGHRP**	**1 vial**
Dilution Buffer - 60 mL of buffered protein based solution with preservative.	**DB09**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB08**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1bottle**
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 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution and Detection Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. ARIGHRP Conjugate

100-fold concentrated solution and TMB Substrate Solution can be stored at 2 – 8° C for up to 6 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components may be stored at 2 – 8° C for up to 6 months.
Microplate Wells: Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

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, 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.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

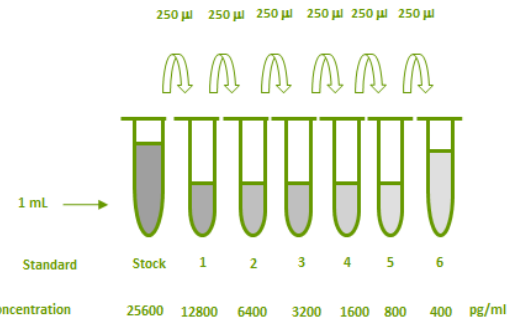
Plasma and serum samples may not require dilution.
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

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 - 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 25600 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 25600 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	25600 pg/ml
# 1	250µl of stock	250µl	12800 pg/ml
# 2	250µl of 1	250µl	6400 pg/ml
# 3	250µl of 2	250µl	3200 pg/ml
# 4	250µl of 3	250µl	1600 pg/ml
# 5	250µl of 4	250µl	800 pg/ml
# 6	250µl of 5	250µl	400 pg/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of **Dilution Buffer**. Positive Control could be reused within a few days if stored at -20° C or -70° C.

Vasostatin-2 Antibody - 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 (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. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µl per well of Dilution Buffer to Blank wells.
4. Add 100 µl per well of standard solution from #6 to #S (reverse order of serial dilution) to the appropriate wells. Add 100 µl per well of Positive control into appropriate wells. Add 100 µl per well of samples into appropriate wells. Seal the plate with plate sealer and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
5. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µl per well of 1x Antibody solution. Seal the plate with plate sealer 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. Seal the plate with plate sealer 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 2-5 minutes on microplate shaker 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 microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and samples, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Vasostatin-2 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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.

STANDARD (PG/ML)	OD450 READING
0 (Blank)	0 (0.154)
400	0.006
800	0.028
1600	0.056
3200	0.131
6400	0.317
12800	0.625
25600	1.301

- Lot No.:
- Positive Control:

SPECIFICITY

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

PROTEIN	CROSSREACTIVITY (%)
Rat Vasostatin-2	100
Human 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 2-5 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.