RAT/MOUSE ACROGRANIN (GRN) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF RAT OR MOUSE ACROGRANIN (GRN) 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.

PURCHASE INFORMATION:

ELISA NAME	**RAT/MOUSE ACROGRANIN ELISA**
Catalog No.	SK00313-03
Formulation	96 T
Lot No.	
Standard range	0.0128 - 200 ng/mL
Sensitivity	0.064 ng/mL
Sample Volume	50 µl
Dilution Factor	5 **(Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	Serum, EDTA Plasma
Specificity	Rat, Mouse, Human
Calibration	Rat/Mouse Acrogranin recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
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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INTRODUCTION

Rat/Mouse Acrogranin ELISA employs the quantitatively competitive enzyme immunoassay technique in which rat/mouse Acrogranin present in samples compete with a fixed amount of biotinylated Acrogranin for sites on purified rabbit IgG specific against Acrogranin. 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 stop solution is added. The intensity of the color measured is in inverse proportion to the amount of rat/mouse Acrogranin bound in the initial step. The sample values are then read off the standard curve.

Rat/Mouse Acrogranin ELISA has been shown to accurately quantify natural rat/mouse Acrogranin. Results obtained using natural rat/mouse Acrogranin showed dose response curves that were parallel to the standard curves obtained using the kit standards.

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 polystyrene microplate (12 strips of 8 wells) coated with polyclonal IgG against rabbit IgG.	**RM01**	**1 plate**
Acrogranin Standard – 200 ng/vial of recombinant Acrogranin in a buffered protein base with preservative; lyophilized.	**313-03-02**	**1 vial**
Antibody Concentrate – 350 µL/vial, 10-fold concentrate of polyclonal purified IgG against Acrogranin with preservative; lyophilized.	**313-03-03**	**1 vial**
Biotin Concentrate - 350 µL/vial, 10-fold concentrate of biotinylated Acrogranin in a buffered protein base with preservative; lyophilized	**313-03-01**	**1 vial**
Positive Control - one vial of recombinant Acrogranin, lyophilized.	**313-03-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of streptavidin conjugate to HRP	**SAHRP**	**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	**DB06**	**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 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° C or -70° C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard, Biotin concentrated solution , Positive Control 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. 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.
- 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.
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.

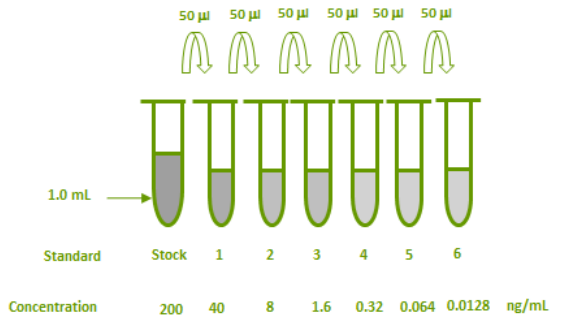
REAGENT PREPARATION

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 1x Wash Buffer.

Acrogranin Standard - Reconstitute the Acrogranin standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 200 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 #6. 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. The Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	200 ng/ml
# 1	50 µl of stock	200 µl	40 ng/ml
# 2	50 µl of 1	200 µl	8 ng/ml
# 3	50 µl of 2	200 µl	1.6 ng/ml
# 4	50 µl of 3	200 µl	0.32 ng/ml
# 5	50 µl of 4	200 µl	0.064 ng/ml
# 6	50 µl of 5	200 µl	0.0128 ng/ml



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 stored at -20° C ~ -70° C).

Antibody Concentrate - Reconstitute the Antibody Concentrate with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 3.15 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 350 µL of 10-fold concentrated stock solution to prepare 1x Antibody working solution.

Biotin Concentrate - Reconstitute the Biotin Concentrate with 350 µL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 3.15 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 350 µL of 10-fold concentrated stock solution to prepare 1x Biotin working solution.

Streptavidin-HRP Conjugate - Transfer 120 µL of 100-fold concentrated stock solution to 11.88 mL of **HRP Diluent Solution (DB06)** to prepare 1x working solution. **Note:** 1x working solution of Streptavidin-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. **DO NOT ADD any Dilution Buffer, Antibody or Biotin Solution to blank wells.**
4. Add 50 µL of Dilution Buffer to Total Binding (TB) wells. Add 50 µL of Standard dilutions, sample, or positive control per well. Add 25 µL of 1x Antibody working solution to each well, excluding blanks. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. **DO NOT aspirate and wash.** Add 25 µL of 1x Biotin working solution to each well, excluding blanks. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
7. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 6.
9. Add 100 µL of Substrate Solution to each well. Incubate for 5-15 minutes on microplate shaker at room temperature. **Protect from light.**
10. 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.
11. 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 sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 Acrogranin 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.

SPECIFICITY

PROTEIN	CROSS-REACTIVITY (%)
Mouse Acrogranin	**100**
Rat Acrogranin	**100**
Human Acrogranin	**100**
Human BDNF	**0**
Human CTGF	**0**
Human SDF-1α	**0**
Human NRG4	**0**
Human FABP7	**0**

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (NG/ML)	CORRECTED (450NM)
Blank	0 (0.071)
Total Binding	1.084
0.0128	1.052
0.064	1.059
0.32	1.127
1.6	1.042
8	0.838
40	0.376
200	0.113

- Lot No.:
- Positive Control:

LINEARITY

To assess the linearity of the assay pooled rat EDTA plasma samples were diluted with Dilution Buffer (DB18) and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
5 x	34.956	174.78	100
10 x	18.722	187.22	107

To assess the linearity of the assay pooled mouse EDTA plasma samples were diluted with Dilution Buffer (DB18) and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
5 x	36.485	182.43	100
10 x	19.374	193.74	106

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 50 µl of standard dilutions, samples, or positive control to the wells. Add 25 µL of 1x Antibody working solution to each well, excluding blanks. Incubate 2 hours on the plate shaker at RT. **DO NOT ASPIRATE OR WASH. PROCEED DIRECTLY TO NEXT STEP.**
↓
Add 25 µl 1x Biotin working solution to each well, excluding blanks. 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 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 5-15 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 minutes.