

MOUSE GAS6 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE GAS6 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, AND PLASMA



PURCHASE INFORMATION:

ELISA NAME	MOUSE GAS6 ELISA
Catalog No.	SK00098-03
Lot No.	
Formulation	96 T
Standard range	39-2500 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µl
Dilution Factor	20 (Optimal dilutions should be determined by each laboratory for each application)
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Mouse Gas6 only
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8°C

Order Contact:
AVISCIERA BIOSCIENCE INC.
 2348 Walsh Ave., Suite C
 Santa Clara, CA 95051
 Tel: (408) 982 0300
 Fax: (408) 982 0301
 Email: Sales@AvisceraBioscience.com
 Info@AvisceraBioscience.com
www.AvisceraBioscience.com

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INTRODUCTION

Mouse Gas6 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure mouse Gas6 in cell culture supernates, serum, and EDTA plasma. It contains recombinant mouse Gas6 and antibodies raised against this protein. It has been shown to accurately quantify recombinant mouse Gas6. Results obtained with naturally occurring Gas6 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural mouse Gas6.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for Gas6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Gas6 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for Gas6 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of Gas6 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ 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 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.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all

factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Gas6 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a goat polyclonal purified IgG against mouse Gas6	098-03-01	1 plate
Gas6 Standard – 2500 pg/vial of recombinant mouse Gas6 in a buffered protein base with preservatives; lyophilized.	098-03-02	1 vial
Detection Antibody – 105µL/vial, 100-fold concentrate of Biotinylated goat polyclonal purified IgG against mouse Gas6 with preservatives; lyophilized.	098-03-03	1 vial
Positive Control – one vial of recombinant mouse Gas6, lyophilized	098-03-04	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	DB01	1 bottle
HRP Diluent Solution – 12 mL of buffered protein based solution with preservatives	DB08	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 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should

be stored at -20 or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-fold concentrate 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 and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

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 be 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 case of contact with skin or eyes wash immediately with water.

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

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

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.

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 require a 20 fold dilution. A suggested 20-fold dilution is 15 µL sample + 285 µL Dilution Buffer. **Optimal dilutions should be determined by each laboratory for each application with a sample pretest.**

Use polypropylene test tubes.

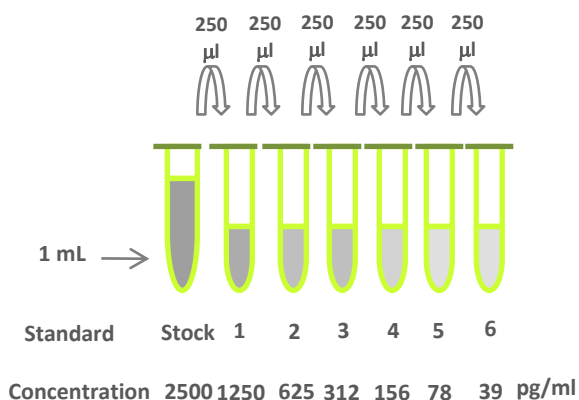
REAGENT PREPARATION

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.

Gas6 Standard - Refer to vial label for reconstitution volume. Reconstitute the Gas6 Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 2500 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 2500 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	2500 pg/ml
# 1	250µl of stock	250µl	1250 pg/ml
# 2	250µl of 1	250µl	625 pg/ml
# 3	250µl of 2	250µl	312 pg/ml
# 4	250µl of 3	250µl	156 pg/ml
# 5	250µl of 4	250µl	78 pg/ml
# 6	250µl of 5	250µl	39 pg/ml



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 105 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock 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. *Note: Positive Control should be prepared and used immediately.*

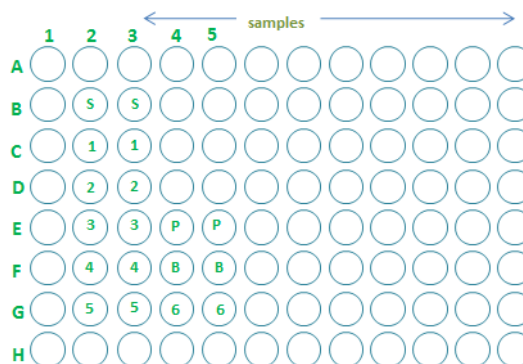
ASSAY PROCEDURE

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. Add 100 µL of Dilution Buffer to Blank well (F4, F5).
4. Add 100 µL of Standard (B2, B3 to G2, G3 and G4, G5), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate

layout is provided to record standards and samples assayed.

5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 min on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 3-7 minutes at room temperature. **Protect from light.**
11. 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 micro-plate 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 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 Gas6 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.

Mouse Gas6 Standard (pg/mL)	Average OD450nm (Corrected)
Blank	0 (0.096)
19 (optional)	0.025
39	0.057
78	0.104
156	0.226
312	0.453
625	0.827
1250	1.515
2500	2.744

*Lot No.: 20110761

** Positive Control: 280-530 pg/mL

CALIBRATION

This immunoassay is calibrated against a highly purified CHO-expressed recombinant Mouse Gas6.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of Gas6 was 10 pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant mouse Gas6. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity.

Proteins	Cross-reactivity
Mouse Gas6	100%
Human Gas6	5.6%
Mouse Gas1	0
Human Gas1	0
Human PROS-1	0

LINEARITY

To assess the linearity of the assay, pooled research Mouse serum samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
20 X	795.904	15918.08	100
40 X	388.121	15524.84	98

To assess the linearity of the assay, pooled research Mouse EDTA plasma samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
20 X	590.541	11810.82	100
40 X	295.360	11814.4	100

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3. Lindqvist PG, et al. Umbilical cord plasma levels of growth-arrest specific protein 6 in intrauterine growth restriction. *Acta Obstet Gynecol Scand.* 2010;89(1):22-6.
4. Ekman C, et al. Plasma concentrations of growth arrest specific protein 6 and the soluble form of its tyrosine kinase receptor Axl as markers of large abdominal aortic aneurysms. *Clin Biochem.* 2010 Jan;43(1-2):110-4. Epub 2009 Aug 4.
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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 Detection Antibody working 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 each well. 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 3-7 min on the bench top. Protect from light.
↓
Add 100 µL Stop Solution to each well. Read 450nm within 15 min

