# Quantikine® ELISA

## Mouse Gas6 Immunoassay

Catalog Number MGAS60

For the quantitative determination of mouse Growth Arrest Specific 6 (Gas6) concentrations in cell culture supernates, cell lysates, serum, and plasma.

**Note: The standard reconstitution method has changed. Read this package insert in its entirety before using this product.**

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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# INTRODUCTION

Gas6 (Growth Arrest Specific 6) is a multi-modular protein that is upregulated by a wide variety of cell types in response to growth arrest. It is expressed by endothelial cells, fibroblasts, neurons, smooth muscle cells, and platelets and plays a role in vascular, thrombotic, atherosclerotic, inflammatory, autoimmune, renal, and cancer pathologies (1-5). Secreted Gas6 is a 75 kDa, 647 amino acid (aa) molecule. Both Gas6 and the related Protein S contain an extensively γ-carboxylated N-terminal Gla domain, four EGF-like repeats, and two C-terminal laminin G-like domains that resemble sex hormone binding globulin (SHBG). In parallel with the structurally-related Protein S, Gas6 is dependent upon Vitamin K for activity. Within the EGF-like and SHBG-like domains, mouse Gas6 shares 83%, 94%, and 40% aa sequence identity with the equivalent regions in human Gas6, rat Gas6, and mouse Protein S, respectively. Gas6 binds to and induces signaling through the receptor tyrosine kinase TAM subfamily, which includes Axl, Dtk/Tyro3, and Mer (2, 6-8). Shed (soluble) forms of Axl and Mer are known to bind Gas6 and function as decoy receptors (9, 10).

Gas6 has a number of divergent functions in tissue that are related to its ability to bind to multiple receptors. Through its Gla and laminin G domains, it participates in tissue homeostasis by protecting cells from stress-induced apoptosis, an effect mediated by binding to Axl, and by promoting apoptotic cell phagocytosis by binding to Mer. In addition, it appears to have neuroprotective properties, an effect possibly mediated through Gas6 binding to either Tyro3 or Axl. Notably, the affinity of the γ-carboxylated Gla domain for phosphatidylserine also likely contributes to its unusual role in phagocytosis as well as the cellular entry of select viruses (11-19). Gas6 may also function as a pro-inflammatory molecule by promoting platelet activation (20-22), the development of nephrotoxic nephritis (23), and atherosclerotic plaque instability (24). Conversely, it can also inhibit inflammatory cytokine production from monocytes/macrophages and microglia (25, 26). Gas6 is known to induce the proliferation of cardiac fibroblasts, Schwann cells, vascular smooth muscle cells, and NK cell precursors (27-30). It also inhibits VEGF-induced endothelial cell chemotaxis (31) and can have either positive or negative effects on the proliferation and invasiveness of tumor cells (14, 32, 33).

Circulating levels of Gas6 are elevated in a variety of conditions including venous thromboembolic disease, systemic lupus erythematosus, and systemic inflammatory responses (34-36). It is also present in the cerebrospinal fluid of patients with chronic inflammatory demyelinating polyneuropathy (CIDP) (37).

The Quantikine® Mouse Gas6 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse Gas6 in cell culture supernates, cell lysates, serum, and plasma. It contains NS0-expressed recombinant mouse Gas6 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant factor accurately. Results obtained using natural mouse Gas6 showed dose-response curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this 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 monoclonal antibody specific for mouse Gas6 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any Gas6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse Gas6 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, 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 Gas6 bound in the initial step. The sample values are then read off the standard curve.

## 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.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent 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.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

| PART | PART # | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|---|
| **Mouse Gas6 Microplate** | 893976 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse Gas6. | Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.* |
| **Mouse Gas6 Standard** | 893978 | 2 vials of recombinant mouse Gas6 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | Discard after use. Prepare fresh for each use. |
| **Mouse Gas6 Control** | 893979 | 2 vials of recombinant mouse Gas6 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label. | |
| **Mouse Gas6 Conjugate** | 893977 | 12 mL of a polyclonal antibody specific for mouse Gas6 conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Assay Diluent RD1-43** | 895521 | 11 mL of a buffered protein solution with blue dye and preservatives. *Contains a precipitate. Mix well before and during use.* | |
| **Calibrator Diluent RD5-26 Concentrate** | 895525 | 21 mL of a concentrated buffered protein base with preservatives. | |
| **Wash Buffer Concentrate** | 895003 | 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.* | |
| **Color Reagent A** | 895000 | 12 mL of stabilized hydrogen peroxide. | |
| **Color Reagent B** | 895001 | 12 mL of stabilized chromogen (tetramethylbenzidine). | |
| **Stop Solution** | 895174 | 23 mL of diluted hydrochloric acid. | |
| **Plate Sealers** | N/A | 4 adhesive strips. | |

* Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.

**If using cell lysate samples, the following is also required:**

- Cell Lysis Buffer 2 (R&D Systems®, Catalog # 895347).

## PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.**

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

**Cell Lysates** - Cells must be lysed prior to assay. See Sample Values section.

**Serum** - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 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 or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Note:** *Citrate plasma has not been validated for use in this assay.*

## SAMPLE PREPARATION

Serum and plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 10 µL of sample + 190 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

*See Reagent Preparation section.

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Mouse Gas6 Control** - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

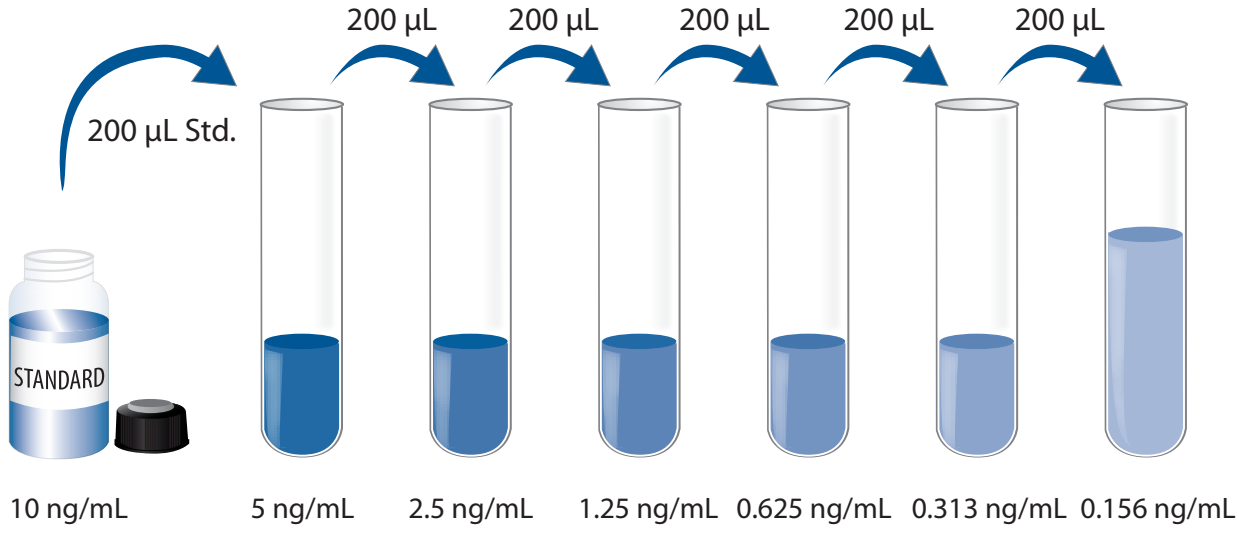
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

**Calibrator Diluent RD5-26 (diluted 1:4)** - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

**Mouse Gas6 Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse Gas6 Standard with Calibrator Diluent RD5-26 (diluted 1:4). This reconstitution produces a stock solution of 10 ng/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-26 (diluted 1:4) into each of six tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse Gas6 Standard (10 ng/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 ng/mL).



## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all samples, control, and standards be assayed in duplicate.**

1. Prepare all reagents, working standards, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-43 to each well. *Assay Diluent RD1-43 contains a precipitate. Mix well before and during use.*
4. Add 50 µL of standard, control, or sample* per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for **3 hours at 2-8 °C.**
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or auto washer. 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 Mouse Gas6 Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour at 2-8 °C.**
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

## CALCULATION OF RESULTS

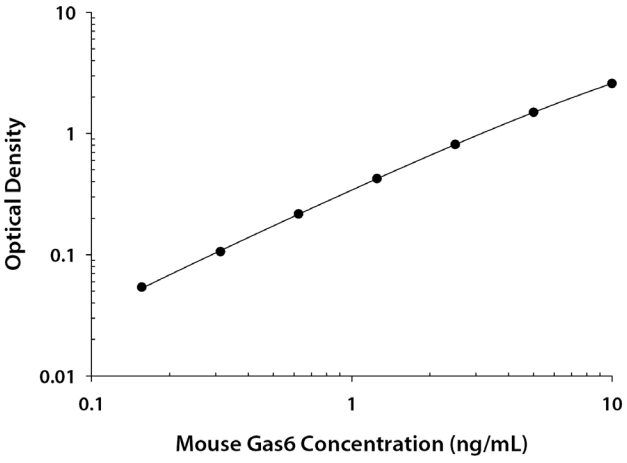
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse 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.




| (ng/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.045<br>0.050 | 0.048 | — |
| 0.156 | 0.100<br>0.102 | 0.101 | 0.053 |
| 0.313 | 0.152<br>0.155 | 0.154 | 0.106 |
| 0.625 | 0.262<br>0.266 | 0.264 | 0.216 |
| 1.25 | 0.469<br>0.474 | 0.472 | 0.424 |
| 2.5 | 0.850<br>0.867 | 0.859 | 0.811 |
| 5 | 1.532<br>1.552 | 1.542 | 1.494 |
| 10 | 2.579<br>2.686 | 2.633 | 2.585 |

## PRECISION

**Intra-assay Precision** (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | 1 | 2 | 3 | 1 | 2 | 3 |
| **n** | 20 | 20 | 20 | 20 | 20 | 20 |
| **Mean (ng/mL)** | 0.451 | 1.28 | 3.93 | 0.465 | 1.30 | 3.98 |
| **Standard deviation** | 0.023 | 0.052 | 0.129 | 0.024 | 0.054 | 0.285 |
| **CV (%)** | 5.1 | 4.1 | 3.3 | 5.2 | 4.2 | 7.2 |

## RECOVERY

The recovery of mouse Gas6 spiked into cell culture samples were evaluated.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture samples (n=4) | 111 | 103-118% |

## LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse Gas6 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture supernates (n=4) | Cell lysates (n=4) | Serum* (n=4) | EDTA plasma* (n=4) | Heparin plasma* (n=4) |
|---|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 97 | 109 | 99 | 102 | 105 |
| | Range (%) | 88-104 | 105-118 | 98-100 | 95-112 | 103-105 |
| 1:4 | Average % of Expected | 97 | 109 | 97 | 105 | 105 |
| | Range (%) | 89-105 | 105-115 | 91-101 | 94-119 | 101-110 |
| 1:8 | Average % of Expected | 100 | 110 | 97 | 104 | 108 |
| | Range (%) | 93-111 | 104-120 | 89-105 | 82-119 | 101-115 |
| 1:16 | Average % of Expected | 104 | 104 | 96 | 103 | 105 |
| | Range (%) | 95-114 | 96-120 | 86-105 | 83-118 | 92-118 |

*Samples were diluted prior to assay.

## SENSITIVITY

Forty-six assays were evaluated and the minimum detectable dose (MDD) of mouse Gas6 ranged from 0.004-0.027 ng/mL. The mean MDD was 0.014 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.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## CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse Gas6 produced at R&D Systems®.

## SAMPLE VALUES

**Serum/Plasma** - Samples were evaluated for the presence of mouse Gas6 in this assay.

| Sample Type | Mean (ng/mL) | Range (ng/mL) | Standard Deviation (ng/mL) |
|---|---|---|---|
| Serum (n=20) | 66.8 | 42.9-96.8 | 14.7 |
| EDTA plasma (n=20) | 46.6 | 33.6-67.9 | 7.0 |
| Heparin plasma (n=20) | 57.6 | 42.4-71.7 | 8.8 |

**Cell Culture Supernates** - Organs from mice were removed, rinsed in 1X PBS, and kept on ice in 1X PBS. Organs were then cut into 1-2 mm pieces and homogenized using a tissue homogenizer. Cells were seeded into media containing RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 1 or 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse Gas6.

| Tissue Type | (ng/mL) |
|---|---|
| Heart (3 days) | 0.442 |
| Kidney (1 day) | 2.61 |
| Spleen (3 days) | 3.88 |

**Cell Lysates** - Organs from mice were rinsed with 1X PBS and homogenized with a tissue homogenizer in 1X PBS. An equal volume of Cell Lysis Buffer 2 was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. An aliquot of each cell lysate was removed and assayed for levels of mouse Gas6.

| Tissue Type | (ng/mL) |
|---|---|
| Heart | 2.46 |
| Kidney | 68.2 |
| Spleen | 103 |

## SPECIFICITY

This assay recognizes natural and recombinant mouse Gas6.

The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant mouse Gas6 control were assayed for interference. No significant cross-reactivity or interference was observed.

**Recombinant mouse:**
Axl
Dtk
Gas1
Mer

**Recombinant human:**
Gas1

**Natural proteins:**
human Protein S

Recombinant human Gas6 cross-reacts approximately 6.5% in this assay.

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