

Quantikine[®] ELISA

Human Gas6 Immunoassay

Catalog Number DGAS60

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

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 one of six structurally unrelated proteins that are upregulated by a wide variety of cell types in response to growth arrest. Gas6 plays a role in vascular, autoimmune, kidney, inflammatory, thrombotic, arteriosclerotic, and cancer pathologies (1-3). The 75 kDa Gas6 and the structurally-related Protein S are vitamin K-dependent and have an extensively γ -carboxylated N-terminal Gla domain, four EGF-like repeats, and a C-terminal region with homology to sex hormone binding globulin (SHBG). There are two potential thrombin cleavage sites between the Gla domain and the first EGF-like domain (4). Alternate splicing of human Gas6 generates isoforms that lack the Gla domain and/or 43 amino acids (aa) of the spacer between the EGF-like and SHBG regions (5, 6). The relative expression levels of the various isoforms is regulated in a tissue-specific manner (5). The isoform-specific 43 aa region contains a protease-sensitive site whose cleavage results in the production of 36 kDa N-terminal and 50 kDa C-terminal fragments (6). This protease-sensitive region is not present in mouse or rat Gas6. Within the EGF-like, spacer, and SHBG domains, human Gas6 shares approximately 84% aa sequence identity with comparable regions of mouse and rat Gas6 and 43% aa identity with human Protein S. Gas6 binds and induces signaling through the receptor tyrosine kinases Axl, Dtk, and Mer (7-9). Human Gas6 interacts with both mouse and rat orthologs of these receptors (1). Shed soluble forms of Axl and Mer bind Gas6 and function as decoy receptors (10, 11). The proteolytically released C-terminal SHBG region of human Gas6 can independently activate Axl (6, 12).

Gas6 functions in tissue homeostasis by protecting cells from stress-induced apoptosis (13-16), promoting the phagocytosis of apoptotic cells (17, 18), and promoting the remyelination of damaged axons (19). The affinity of Gas6 for phosphatidylserine contributes to its role in apoptotic cell phagocytosis and also the cellular entry of lentiviruses (20-22). The anti-apoptotic, phagocytic, and viral entry functions have been shown to require γ -carboxylation of the Gla domain (17, 22, 23). Gas6 functions as a pro-inflammatory molecule by promoting platelet-mediated thrombosis (24-26), the development of nephrotoxic nephritis (27), and atherosclerotic plaque instability (28), but it can also inhibit inflammatory cytokine production from monocytes/macrophages and microglia (29, 30). Gas6 promotes the proliferation of cardiac fibroblasts, Schwann cells, vascular smooth muscle cells, and NK cell precursors (31-34). It inhibits VEGF-induced endothelial cell chemotaxis (35) and can have either positive or negative effects on the proliferation and invasiveness of tumor cells (16, 36, 37). Gas6 is released by Epo-stimulated erythroblasts and enhances signaling through the Epo R (38).

Circulating levels of Gas6 are elevated in a variety of conditions including venous thromboembolic disease, systemic lupus erythematosus, and systemic inflammatory responses (39-41) as well as in the cerebrospinal fluid of patients with chronic inflammatory demyelinating polyneuropathy (CIDP) and non-inflammatory/non-autoimmune neurological disease (NINAD) (42). Gas6 expression is normally positively correlated with the levels of soluble Mer and Axl but are negatively correlated with these receptors in multiple sclerosis lesions (43).

The Quantikine Human Gas6 immunoassay is a 4.5 hour solid phase ELISA designed to measure human Gas6 in cell culture supernates, serum, plasma, and urine. It contains NS0-expressed recombinant human Gas6 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural Gas6 showed linear 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 naturally occurring human Gas6.

PRINCIPLE OF THE ASSAY

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

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 the appropriate 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 soluble receptors, binding proteins, and 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.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate 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
Gas6 Microplate	893387	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against human Gas6.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Gas6 Conjugate	893388	21 mL of a goat polyclonal antibody against human Gas6 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Gas6 Standard	893389	40 ng of recombinant human Gas6 in a buffered protein solution with preservatives; lyophilized.	
Assay Diluent RD1-43	895521	11 mL of a buffered protein solution with preservatives. <i>Contains a precipitate. Mix well before and during use.</i>	
Calibrator Diluent RD6-10	895468	21 mL of a buffered protein solution with preservatives. <i>For cell culture supernate/urine samples.</i>	
Calibrator Diluent RD6-54	895945	21 mL of a buffered protein solution with preservatives. <i>For serum/plasma samples.</i>	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservatives.	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric 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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human Gas6 Controls (optional; available from R&D Systems).

PRECAUTIONS

Calibrator Diluent RD6-10 contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

Gas6 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Cell culture supernate samples require a 2-fold dilution. A suggested 2-fold dilution is 200 μ L of sample + 200 μ L of Calibrator Diluent RD6-10 (1:5).

Serum and plasma samples require a 5-fold dilution. A suggested 5-fold dilution is 50 μ L of sample + 200 μ L of Calibrator Diluent RD6-54.

Urine samples require a 10-fold dilution. A suggested 10-fold dilution is 50 μ L of sample + 450 μ L of Calibrator Diluent RD6-10 (1:5).

REAGENT PREPARATION

Bring all reagents to room temperature before use..

Note: *Gas6 is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

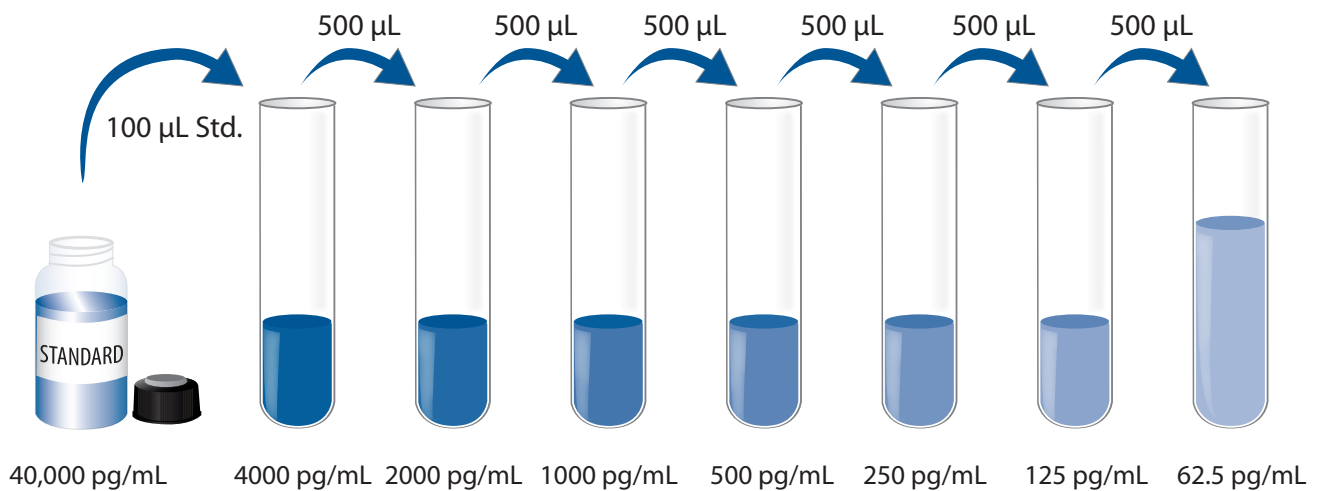
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD6-10 (1:5) - Dilute 4 mL of Calibrator Diluent RD6-10 into 16 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD6-10 (1:5).

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μ L of the resultant mixture is required per well.

Gas6 Standard - Reconstitute the Gas6 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 40,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 μ L of Calibrator Diluent RD6-10 (1:5) (*for cell culture supernate/urine samples*) or Calibrator Diluent RD6-54 (*for serum/plasma samples*) into the 4000 pg/mL tube. Pipette 500 μ L of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

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

Note: *Gas6 is detectable in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, 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 100 μL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. 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 (400 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 200 μL of Gas6 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μL of Substrate Solution to each well.
For cell culture supernate/urine samples: Incubate for 20 minutes at room temperature on the benchtop. **Protect from light.**
For serum/plasma samples: Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 50 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 the color change does not appear uniform, 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 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.).

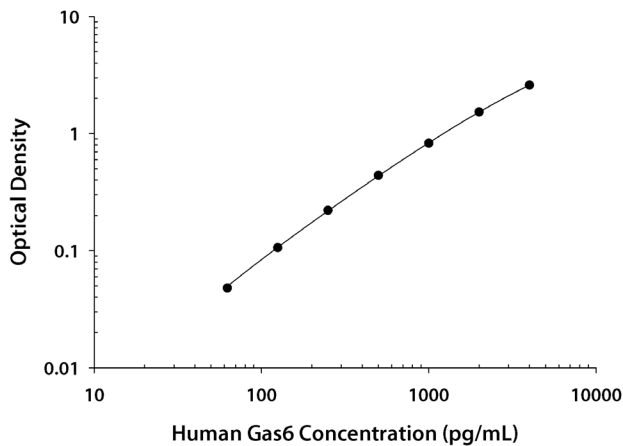
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the 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.

Since samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

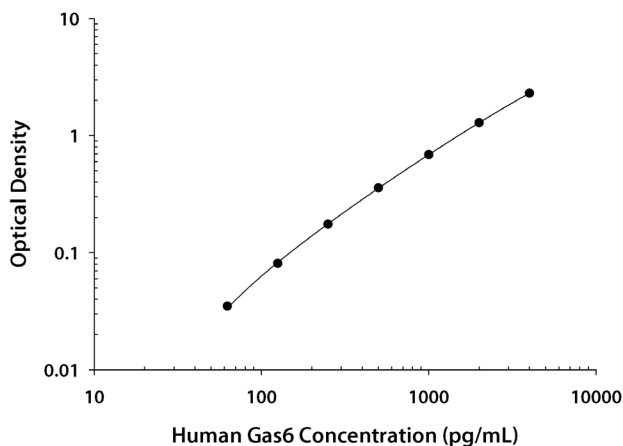
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CALIBRATOR DILUENT RD6-10 (1:5)



(pg/mL)	O.D.	Average	Corrected
0	0.005 0.006	0.006	—
62.5	0.054 0.054	0.054	0.048
125	0.109 0.114	0.112	0.106
250	0.224 0.229	0.227	0.221
500	0.439 0.450	0.445	0.439
1000	0.825 0.841	0.833	0.827
2000	1.533 1.537	1.535	1.529
4000	2.587 2.602	2.595	2.589

CALIBRATOR DILUENT RD6-54



(pg/mL)	O.D.	Average	Corrected
0	0.008 0.009	0.009	—
62.5	0.043 0.044	0.044	0.035
125	0.089 0.091	0.090	0.081
250	0.182 0.186	0.184	0.175
500	0.357 0.375	0.366	0.357
1000	0.675 0.716	0.696	0.687
2000	1.289 1.303	1.296	1.287
4000	2.307 2.312	2.310	2.301

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 forty separate assays to assess inter-assay precision.

CELL CULTURE SUPERNATE/URINE ASSAY

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	346	737	1596	370	777	1609
Standard deviation	16.7	24.4	55.9	28.6	61.2	104
CV (%)	4.8	3.3	3.5	7.7	7.9	6.5

SERUM/PLASMA ASSAY

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	415	936	2015	399	867	1817
Standard deviation	29.3	55.8	68.5	34.9	69.0	154
CV (%)	7.1	6.0	3.4	8.7	8.0	8.5

RECOVERY

The recovery of human Gas6 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates* (n=4)	106	99-110%
Serum* (n=4)	100	90-109%
EDTA plasma* (n=4)	98	83-111%
Heparin plasma* (n=4)	102	95-111%
Urine* (n=4)	96	84-104%

*Samples were diluted prior to assay.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Gas6 were serially diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media* (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	96	99	101	98	100
	Range (%)	90-102	96-100	97-105	96-100	98-103
1:4	Average % of Expected	91	96	101	98	100
	Range (%)	88-93	91-99	100-104	95-102	97-106
1:8	Average % of Expected	91	96	103	98	100
	Range (%)	89-94	91-102	101-106	93-104	95-108
1:16	Average % of Expected	92	96	102	98	100
	Range (%)	89-95	90-99	98-104	92-104	95-110

*Samples were diluted prior to assay.

SENSITIVITY

Sixty-five assays were evaluated and the minimum detectable dose (MDD) of human Gas6 ranged from 0.78-7.70 pg/mL. The mean MDD was 2.29 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

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

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human Gas6 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum* (n=36)	6474	3904-10,162	1668
EDTA plasma* (n=36)	6584	3915-10,639	1843
Heparin plasma* (n=36)	6551	3957-10,256	1741

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Urine* (n=10)	1255	60	ND-1884

ND=Non-detectable

Cell Culture Supernates* -

Human peripheral blood leukocytes (PBLs) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 24 hours or 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of natural human Gas6. All samples measured below the lowest standard, 62.5 pg/mL.

MDA-MB-231 human breast cancer cells were cultured in Leibovitz's L-15 media with 10% fetal bovine serum and 2 mM L-glutamine in a 37 °C CO₂-free incubator. An aliquot of the cell culture supernate was removed, assayed for levels of natural human Gas6, and measured 965 pg/mL.

IMR-90 human lung fibroblasts were cultured in MEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 0.1 mM sodium pyruvate. An aliquot of the cell culture supernate was removed, assayed for levels of natural human Gas6, and measured 2802 pg/mL.

SW1353 human chondrosarcoma cells were cultured in Leibovitz's L-15 media with 10% fetal bovine serum, 2 mM L-glutamine, and stimulated with 10 ng/mL of recombinant human IL-1β in a 37 °C CO₂-free incubator. An aliquot of the cell culture supernate was removed, assayed for levels of natural human Gas6, and measured 232 pg/mL.

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SPECIFICITY

This assay recognizes natural and recombinant human Gas6.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Gas6 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Axl
Dtk
Gas1
LAP
Mer

Recombinant mouse:

Axl
Dtk
Gas1
Gas6
Mer

Natural proteins:

Protein S

REFERENCES

1. Hafizi, S. and B. Dahlback (2006) FEBS J. **273**:5231.
2. Fernandez-Fernandez-L. *et al.* (2008) Thromb. Haemost. **100**:604.
3. Bellido-Martin, L. and P.G. de Frutos (2008) Vitam. Horm. **78**:185.
4. Manfioletti, G. *et al.* (1993) Mol. Cell Biol. **13**:4976.
5. Marcandalli, P. *et al.* (1997) FEBS Lett. **415**:56.
6. Goruppi, S. *et al.* (1997) FEBS Lett. **415**:59.
7. Stitt, T.N. *et al.* (1995) Cell **80**:661.
8. Nagata, K. *et al.* (1996) J. Biol. Chem. **271**:30022.
9. Varnum, B.C. *et al.* (1995) Nature **373**:623.
10. Sather, S. *et al.* (2007) Blood **109**:1026.
11. Ekman, C. *et al.* (2010) J. Thromb. Haemost. **8**:838.
12. Mark, M.R. *et al.* (1996) J. Biol. Chem. **271**:9785.
13. Shankar, S.L. *et al.* (2006) J. Neurosci. **26**:5638.
14. Scutera, S. *et al.* (2009) J. Immunol. **183**:3004.
15. Shiozawa, Y. *et al.* (2010) Exp. Hematol. **38**:132.
16. Shiozawa, Y. *et al.* (2010) Neoplasia **12**:116.
17. Hall, M.O. *et al.* (2002) Exp. Eye Res. **75**:391.
18. Karl, M.O. *et al.* (2008) Cell. Signal. **20**:1159.
19. Binder, M.D. *et al.* (2011) PLoS ONE **6**:e17727.
20. Wu, Y. *et al.* (2005) J. Cell Sci. **118**:539.
21. Xiong, W. *et al.* (2008) Reproduction **135**:77.
22. Morizono, K. *et al.* (2011) Cell Host Microbe **9**:286.
23. Hasanbasic, I. *et al.* (2005) J. Thromb. Haemost. **3**:2790.
24. Gould, W.R. *et al.* (2005) J. Thromb. Haemost. **3**:733.
25. Tjwa, M. *et al.* (2008) Blood **111**:4096.
26. Cosemans, J.M.E.M. *et al.* (2010) J. Thromb. Haemost. **8**:1797.
27. Yanagita, M. *et al.* (2002) J. Clin. Invest. **110**:239.
28. Lutgens, E. *et al.* (2008) J. Pathol. **216**:55.
29. Alciato, F. *et al.* (2010) J. Leukoc. Biol. **87**:869.
30. Grommes, C. *et al.* (2008) J. Neuroimmune Pharmacol. **3**:130.
31. Stenhoff, J. *et al.* (2004) Biochem. Biophys. Res. Commun. **319**:871.
32. Li, R. *et al.* (1996) J. Neurosci. **16**:2012.
33. Nakano, T. *et al.* (1995) J. Biol. Chem. **270**:5702.
34. Caraux, A. *et al.* (2006) Nat. Immunol. **7**:747.
35. Gallicchio, M. *et al.* (2005) Blood **105**:1970.
36. Loges, S. *et al.* (2010) Blood **115**:2264.
37. Gustafsson, A. *et al.* (2009) PLoS ONE **4**:e7575.
38. Angelillo-Scherrer, A. *et al.* (2008) J. Clin. Invest. **118**:583.
39. Blostein, M.D. *et al.* (2011) J. Thromb. Thrombolysis **32**:272.
40. Ekman, C. *et al.* (2011) Rheumatology **50**:1064.
41. Ekman, C. *et al.* (2010) Crit. Care **14**:R158.
42. Sainaghi, P.P. *et al.* (2008) J. Neurol. Sci. **269**:138.
43. Weinger, J.G. *et al.* (2009) Am. J. Pathol. **175**:28

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

12									
11									
10									
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	A	B	C	D	E	F	G	H	

NOTES

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