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R&D SYSTEMS

Saliva Sensitive

Quantikine® ELISA

Human Galectin-9 Immunoassay

Catalog Number DGAL90

For the quantitative determination of human Galectin-9 concentrations in cell culture supernates, serum, plasma, saliva, urine, and human milk.

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

Galectins comprise a family of multifunctional carbohydrate-binding proteins with specificity for N-acetyl-lactosamine-containing glycoproteins. There are at least 14 mammalian Galectins that share structural similarities in their carbohydrate recognition domains (CRD), forming three groups: prototype (one CRD), tandem-repeat (two CRDs), and chimeric (one CRD, unique N-terminus) (1-3). Full length Galectin-9 is a widely expressed 39 kDa tandem-repeat Galectin that contains two CRDs connected by a linker region (4, 5). Progressive deletion within the linker region generates a 36 kDa isoform, also known as Ecalectin or Urate Transporter (UAT) and a 35 kDa isoform (6, 7). The Ecalectin isoform of human Galectin-9 shares 70% and 73% amino acid (aa) sequence identity with the corresponding regions of mouse and rat Galectin-9, respectively.

Galectin-9 exhibits a wide range of activities in the immune system through its interactions with the carbohydrates on multiple proteins including TIM-3, IgE, and CD40 (8-14). Galectin-9 promotes Th2 biased immune responses, expansion of regulatory T cells (Treg) and memory T cells, and the maturation of dendritic cells (10, 15-20). It inhibits the development or activity of Th1, Th17, NK, NKT, and $CD8^+$ cytotoxic T cells (10, 15-18, 21-23). Galectin-9 suppresses immune complex induced inflammation by modulating Fc receptor expression on macrophages and by preventing IgE immune complex formation (9, 24). It limits disease severity in inflammatory disorders such as high fat diet-induced liver disease, collagen-induced arthritis, viral myocarditis, EAE, and rheumatoid arthritis (15, 16, 18, 23, 25). It also promotes the resolution of inflammation by promoting macrophage clearance of mycobacteria infected cells (26). Galectin-9 additionally functions as an eosinophil chemoattractant (27). This activity is destroyed by thrombin-mediated cleavage within the linker region of the long isoform, although the Ecalectin isoform is resistant to thrombin (28). In cancer, Galectin-9 suppresses tumor cell metastasis by interfering with the associations between hyaluronic acid and CD44 and between VCAM-1 and Integrin α4β1 (29). The Ecalectin isoform (UAT) can also be expressed as an integral membrane protein in renal epithelial cells where it mediates the cellular efflux of urate (30).

The Quantikine® Human Galectin-9 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Galectin-9 in cell culture supernates, serum, plasma, saliva, urine, and human milk. It contains recombinant human Galectin-9 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Galectin-9 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 Galectin-9.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Galectin-9 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Galectin-9 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Galectin-9 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 Galectin-9 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 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
Human Galectin-9 Microplate	894648	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Galectin-9.	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.*
Human Galectin-9 Conjugate	894649	21 mL of a polyclonal antibody specific for human Galectin-9 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Galectin-9 Standard	894650	Recombinant human Galectin-9 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-9	895167	11 mL of a buffered protein base with preservatives. *May contain a precipitate. Warm to room temperature, and mix gently to dissolve. If the precipitate does not completely dissolve, mix well during use.*	
Calibrator Diluent RD6-35	895360	21 mL of diluted animal serum with preservatives. *Use undiluted for serum/ plasma/ urine assay. Use diluted 1:4 for cell culture supernate/saliva/human milk assay.*	
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	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.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human Galectin-9 Controls (optional; Catalog # QC96).

PRECAUTIONS

Galectin-9 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

Calibrator Diluent RD6-35 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.

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 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 at room temperature 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.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 100 µL of sample + 300 µL of Calibrator Diluent RD6-35.

Urine samples require a 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calibrator Diluent RD6-35.

Human milk samples require a 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calibrator Diluent RD6-35 (diluted 1:4).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *Galectin-9 is found 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. Add 20 mL of Wash Buffer Concentrate to 480mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

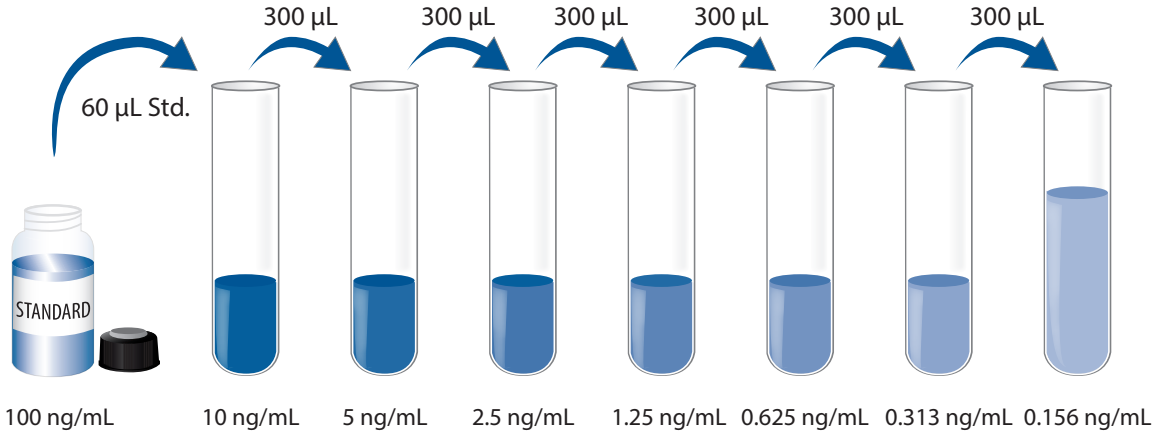
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD6-35 (diluted 1:4) - **For use with cell culture supernate/saliva/human milk samples.** Add 5 mL of Calibrator Diluent RD6-35 to 15 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD6-35 (diluted 1:4).

Note: *Do not dilute Calibrator Diluent RD6-35 for use with serum/plasma/urine samples.*

Human Galectin-9 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Galectin-9 Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 540 µL of Calibrator Diluent RD6-35 (*for serum/plasma/urine samples*) or Calibrator Diluent RD6-35 (diluted 1:4) (*for cell culture supernate/saliva/human milk samples*) into the 10 ng/mL tube. Pipette 300 µL 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 10 ng/mL standard serves as the high standard. The appropriate calibrator diluent 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, controls, and standards be assayed in duplicate.

Note: *Galectin-9 is found 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 100 µL of Assay Diluent RD1-9 to each well. *Assay Diluent RD1-9 may contain 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 the 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 Human Galectin-9 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. **Protect from light.**
   For cell culture supernate/saliva/human milk samples: Incubate for 20 minutes at room temperature **on the benchtop.**
   For serum/plasma/urine samples: Incubate for 30 minutes at room temperature **on the benchtop.**
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 may require dilution. See the 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.).

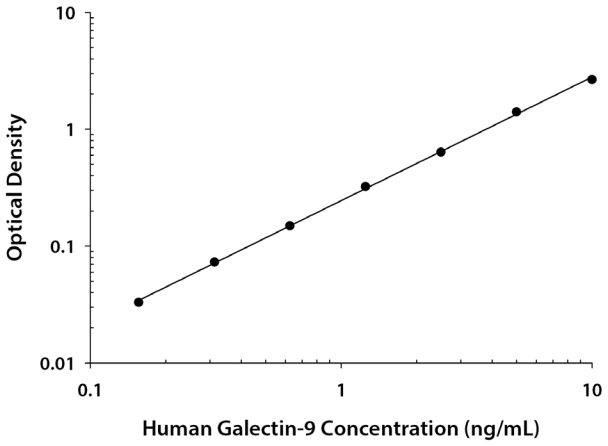
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human Galectin-9 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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

TYPICAL DATA

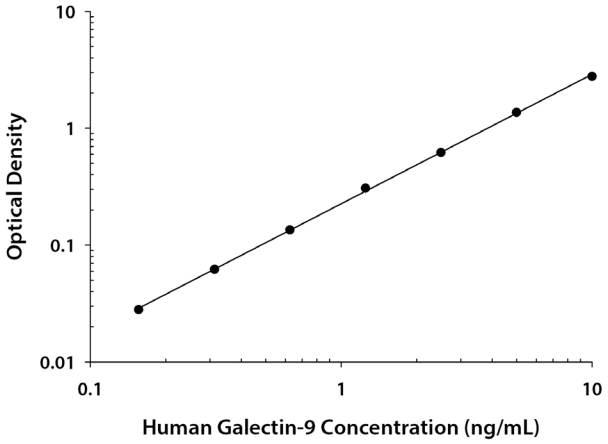
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/SALIVA/HUMAN MILK ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.008 0.008	0.008	—
0.156	0.040 0.042	0.041	0.033
0.313	0.079 0.082	0.081	0.073
0.625	0.155 0.159	0.157	0.149
1.25	0.325 0.336	0.331	0.323
2.5	0.635 0.655	0.645	0.637
5	1.381 1.442	1.412	1.404
10	2.605 2.725	2.665	2.657

SERUM/PLASMA/URINE ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.009 0.009	0.009	—
0.156	0.036 0.037	0.037	0.028
0.313	0.071 0.071	0.071	0.062
0.625	0.143 0.145	0.144	0.135
1.25	0.307 0.325	0.316	0.307
2.5	0.629 0.631	0.630	0.621
5	1.363 1.380	1.372	1.363
10	2.775 2.788	2.782	2.773

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.

CELL CULTURE SUPERNATE/SALIVA/HUMAN MILK ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (ng/mL)	1.34	3.58	6.13	1.44	3.43	6.05
Standard deviation	0.05	0.12	0.22	0.08	0.18	0.32
CV (%)	3.7	3.4	3.6	5.6	5.2	5.3

SERUM/PLASMA/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (ng/mL)	1.44	3.77	6.29	1.40	3.28	5.84
Standard deviation	0.06	0.07	0.08	0.11	0.20	0.30
CV (%)	4.2	1.9	1.3	7.9	6.1	5.1

RECOVERY

The recovery of human Galectin-9 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	96	84-104%
Serum* (n=4)	106	86-117%
EDTA plasma* (n=4)	103	85-117%
Heparin plasma* (n=4)	104	89-117%

*Samples were diluted prior to assay.

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human Galectin-9 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Saliva (n=4)	Urine* (n=4)	Human milk* (n=4)
1:2	Average % of Expected	107	110	111	110	113	101	106
	Range (%)	99-111	105-116	105-118	106-116	108-122	93-111	102-111
1:4	Average % of Expected	103	111	112	109	108	92	107
	Range (%)	98-108	105-117	106-117	104-116	106-111	87-97	106-109
1:8	Average % of Expected	102	104	99	95	107	92	109
	Range (%)	96-108	95-112	90-106	84-103	99-112	89-97	107-114
1:16	Average % of Expected	105	103	92	91	———	94	105
	Range (%)	96-115	91-117	87-105	82-100	———	85-98	100-114

*Samples were diluted prior to assay.

SENSITIVITY

Fifty assays were evaluated and the minimum detectable dose (MDD) of human Galectin-9 ranged from 0.003-0.028 ng/mL. The mean MDD was 0.008 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.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human Galectin-9 manufactured at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of human Galectin-9 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=36)	7.00	3.10-10.4	1.63
EDTA plasma (n=36)	6.97	3.57-10.9	1.68
Heparin plasma (n=36)	6.94	3.34-11.0	1.68
Saliva (n=10)	1.09	0.379-2.00	0.612
Urine (n=11)	50.6	23.7-99.2	22.3
Human milk (n=8)	54.6	11.5-125	39.4

Cell Culture Supernates:
Human peripheral blood mononuclear cells were cultured in DMEM supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of human Galectin-9.

Condition	Day 1 (ng/mL)	Day 6 (ng/mL)
Unstimulated	0.195	0.304
Stimulated	1.05	7.01

KATO-III human gastric carcinoma cells were cultured in IMDM supplemented with 20% fetal bovine serum and 2 mM L-glutamine in 5% CO_2. An aliquot of the cell culture supernate was removed, assayed for human Galectin-9, and measured 0.823 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Galectin-9.

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 human Galectin-9 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CD44v2
Galectin-1
Galectin-2
Galectin-3
Galectin-4
Galectin-7
Galectin-8
Galectin-10
Galectin-14
Integrin α4β1
TIM-3
VCAM-1

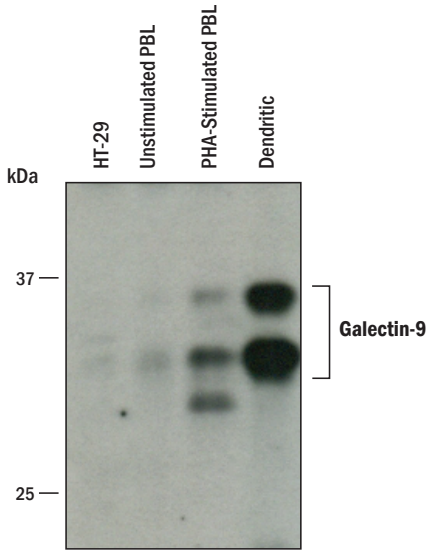
Recombinant mouse:

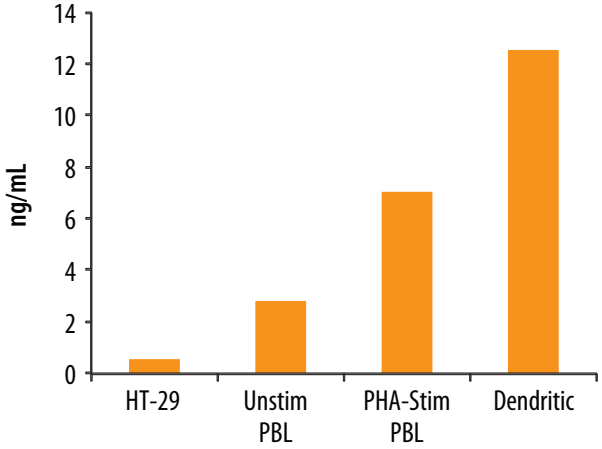
Galectin-1
Galectin-2
Galectin-3
Galectin-4
Galectin-7

Other factors:

human IgE
S. Pyogenes Hyaluronan (Ultra Low MW)
S. Pyogenes Hyaluronan (Low MW)
S. Pyogenes Hyaluronan (Medium MW)
S. Pyogenes Hyaluronan (High MW)
Lactose
Sucrose

Recombinant mouse Galectin-9 cross-reacts approximately 1.5% in this assay.





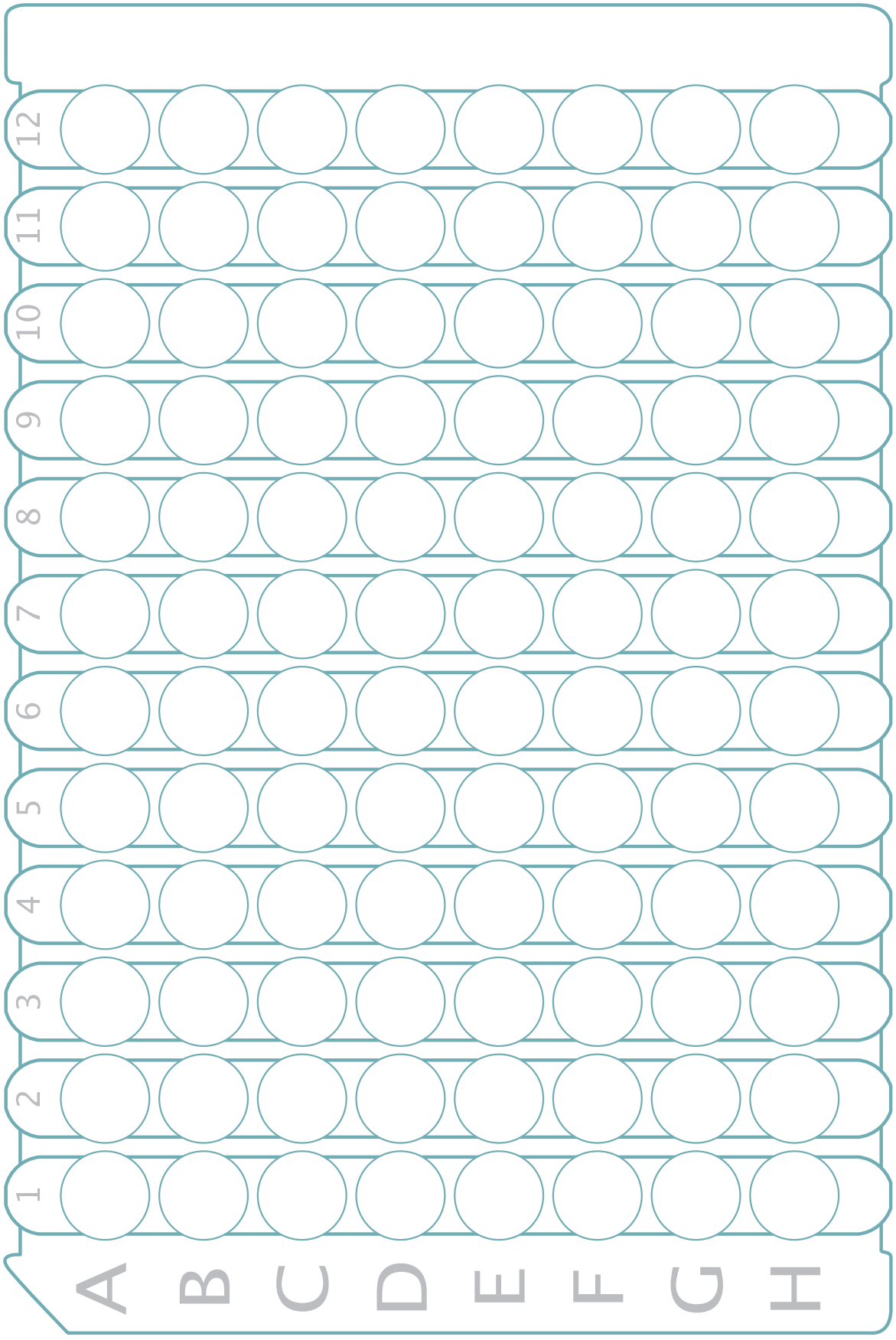
Cell culture media from the indicated cells were analyzed by Western blot and Quantikine® ELISA. Media samples were resolved under reducing SDS-PAGE conditions, transferred to a PVDF membrane, and immunoblotted with anti-hGalectin-9. The Western blot band intensity shows a direct correlation with ELISA sample values. The approximate MW of the bands reflects the presence of both the full length and short forms of hGalectin-9, 40 and 36 kDa, respectively.

REFERENCES

1. Yang, R.Y. *et al.* (2008) Expert Rev. Mol. Med. **10**:e17.
2. Elola, M. T. *et al.* (2007) Cell. Mol. Life Sci. **64**:1679.
3. Zhu, C. *et al.* (2011) Curr. Top. Microbiol. Immunol. **350**:1.
4. Tureci, O. *et al.* (1997) J. Biol. Chem. **272**:6416.
5. Wada, J. and Y.S. Kanwar (1997) J. Biol. Chem. **272**:6078.
6. Chabot, S. *et al.* (2002) Glycobiology **12**:111.
7. Matsumoto, R. *et al.* (1998) J. Biol. Chem. **273**:16976.
8. Leitner, J. *et al.* (2013) PLoS Pathogens **9**:e1003253.
9. Niki, T. *et al.* (2009) J. Biol. Chem. **284**:32344.
10. Zhu, C. *et al.* (2005) Nat. Immunol. **6**:1245.
11. Su, E.W. *et al.* (2011) Glycobiology **21**:1258.
12. Vaitaitis, G.M. and D.H. Wagner (2012) PLoS ONE **7**:e38708.
13. Nagae, M. *et al.* (2009) Glycobiology **19**:112.
14. Sato, M. *et al.* (2002) Glycobiology **12**:191.
15. Lv, K. *et al.* (2011) Clin. Immunol. **140**:92.
16. Seki, M. *et al.* (2008) Clin. Immunol. **127**:78.
17. Sehrawat, S. *et al.* (2010) PloS Pathogens **6**:e1000882.
18. Oomizu, S. *et al.* (2012) Clin. Immunol. **143**:51.
19. Gooden, M.J.M. *et al.* (2013) PLoS ONE **8**:e65616.
20. Dai, S.Y. *et al.* (2005) J. Immunol. **175**:2974.
21. Oomizu, S. *et al.* (2012) PLoS ONE **7**:e48574.
22. Golden-Mason, L. *et al.* (2013) J. Virol. **87**:4835.
23. Tang, Z.-H. *et al.* (2013) J. Immunol. **190**:1788.
24. Arikawa, T. *et al.* (2009) Clin. Immunol. **133**:382.
25. Seki, M. *et al.* (2007) Arthritis Rheum. **56**:3968.
26. Jayaraman, P. *et al.* (2010) J. Exp. Med. **207**:2343.
27. Matsumoto, R. *et al.* (2002) J. Immunol. **168**:1961.
28. Nishi, N. *et al.* (2006) Glycobiology **16**:15C.
29. Nobumoto, A. *et al.* (2008) Glycobiology **18**:735.
30. Leal-Pinto, E. *et al.* (2002) Am. J. Physiol. Renal Physiol. **283**:F150.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES