Quantikine® ELISA

Human Galectin-1 Immunoassay

Catalog Number DGAL10

For the quantitative determination of human Galectin-1 concentrations in cell culture supernates, serum, plasma, saliva, 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

The galectins constitute a large family of carbohydrate-binding proteins with specificity for N-acetyl-lactosamine-containing glycoproteins. The galectins have been classified into the prototype galectins (-1, -2, -5, -7, -10, -11, -13, -14), which contain one carbohydrate recognition domain (CRD) and exist either as a monomer or a noncovalent homodimer; the chimera galectin (galectin-3) containing one CRD linked to a nonlectin domain; and the tandem-repeat galectins (-4, -6, -8, -9, -12) consisting of two CRDs joined by a linker peptide (1, 2). Galectins lack a classical signal peptide and can be localized to the cytosolic compartments where they have intracellular functions. However, galectins can also be secreted via non-classical pathways to function extracellularly. Galectins vary in their tissue distribution profiles and exhibit subtle differences in their carbohydrate-binding specificities (3). Galectin-1 specificity depends on glycan structure, modifications such as sialylation, manner of presentation, and its own monomer/dimer structure and concentration, and may thus have pleiotropic and cell type-specific effects (2-6). It preferentially binds laminin, fibronectin, 90K/Mac-2BP, CD45, CD43, CD7, CD2, CD3, integrins α4β1, α5β1 and α4β7, and ganglioside GM1 (2, 4). Secreted Galectin-1 has immunosuppressive and anti-inflammatory properties and suppresses acute and chronic inflammation and autoimmunity. It contributes to negative selection of developing T cells, immunosuppression by regulatory T cells, resolution of the inflammatory response, and inhibition of immune cell migration, inflammatory cytokine production, and mast cell degranulation (1, 2, 6-8).

Galectin-1, gene name LGALS1 (lectin, galactoside-binding, soluble 1), is a non-glycosylated monomeric or homodimeric prototype galectin that is expressed in a variety of tissues, including smooth and striated muscle, kidney, liver, intestine, prostate, lymph nodes, spleen, thymus, placenta, testis, retina (2, 4, 13, 14). It is produced by cells including endothelial cells, connective tissue fibroblasts, thymic stromal cells, tumor cells, muscle cells, platelets, regulatory T cells, and activated tissue macrophages, B cells, T cells and dendritic cells (2, 4, 8-13). Most of this expression is cytosolic. Endothelial cell surface expression, including tumor endothelial cells, is greatly increased by cell activation (11). Galectin-1 is highly expressed at the maternal-fetal interface and contributes to fetal immune privilege (7, 14). Its immunosuppressive properties appear to also allow tumor cells to evade immune detection (6, 7). It selectively controls T cell survival by inducing apoptosis of activated Th1 and Th17 cells, which express Galectin-1-binding glycans, while promoting Th2 cell survival where glycans are sialylated and less recognized (6, 15). It also induces apoptosis of immature thymocytes (4, 8). Galectin-1 secreted from bone marrow stromal cells aids B lymphocyte development by contributing to pre-B cell integrin adhesion and receptor signaling (4). The dimer form of Galectin-1 also induces neutrophil downregulation by inducing cell surface exposure of phosphatidylserine that marks the cell for phagocytosis (16). Galectin-1 can also modulate cell-cell and cell-matrix interactions, and can promote either cell attachment or detachment depending on the cell type and developmental stage (1, 2). Increased circulating Galectin-1 has been reported in type 2 diabetes and pre-surgical colorectal carcinomas (17, 18).

The Quantikine Human Galectin-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Galectin-1 in cell culture supernates, serum, plasma, saliva, and human milk. It contains *E. coli*-expressed recombinant human Galectin-1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Galectin-1 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 Galectin-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for Galectin-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Galectin-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for Galectin-1 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-1 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 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
Galectin-1 Microplate	893400	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against Galectin-1.	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.*
Galectin-1 Conjugate	893401	21 mL of a polyclonal antibody against Galectin-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Galectin-1 Standard	893402	80 ng of recombinant human Galectin-1 in a buffer with preservatives; lyophilized.	
Assay Diluent RD1-9	895167	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-24	895325	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	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-1 Controls (optional; available from R&D Systems).

PRECAUTIONS

Galectin-1 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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

Some components in this kit contain ProClin® 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. Please 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.*
Icteric samples are not recommended 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.

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

Cell culture supernate, saliva, and human milk samples require at least a 2-fold dilution. A suggested 2-fold dilution is 150 µL of sample + 150 µL of Calibrator Diluent RD5-24 (1:10).

Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 40 µL of sample + 360 µL of Calibrator Diluent RD5-24 (1:10).

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of Galectin-1 are 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 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.

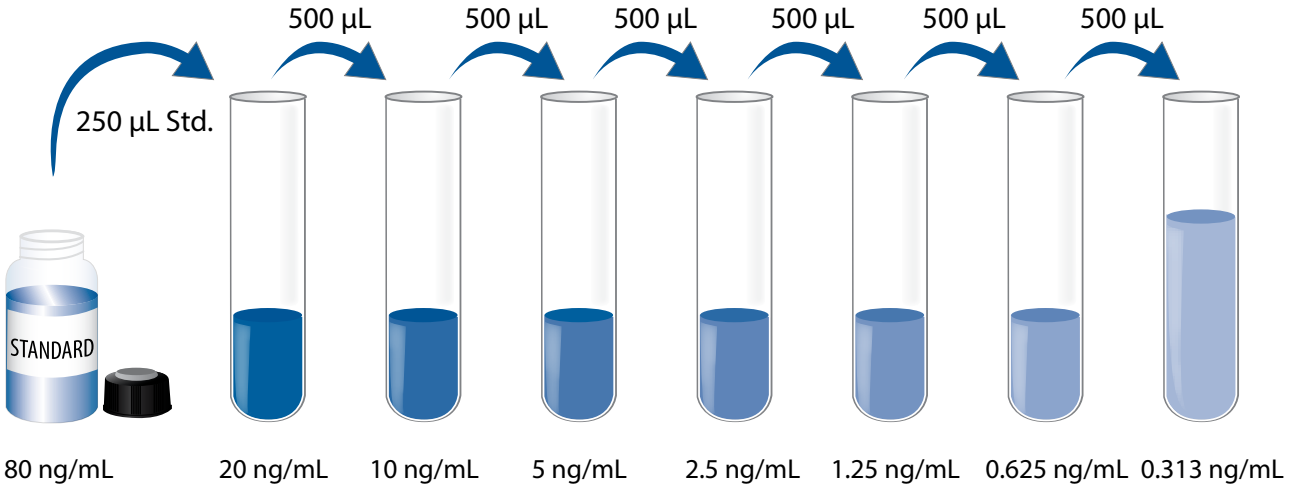
Calibrator Diluent RD5-24 (1:10) - Add 2 mL of Calibrator Diluent RD5-24 to 18 mL of deionized or distilled water to yield 20 mL of Diluted Calibrator Diluent RD5-24 (1:10).

Note: *Calibrator Diluent RD5-24 (1:10) cannot be stored once diluted.*

Galectin-1 Standard - Reconstitute the Galectin-1 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 80 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 750 µL of Calibrator Diluent RD5-24 (1:10) into the 20 ng/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20 ng/mL standard serves as the high standard. Calibrator Diluent RD5-24 (1:10) serves as the zero standard (0 ng/mL).

Note: *Standard curve must be added to the plate within 30 minutes of preparation.*



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: *High concentrations of Galectin-1 are 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.
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.
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 Galectin-1 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. 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 the Sample Preparation section.

CALCULATION OF RESULTS

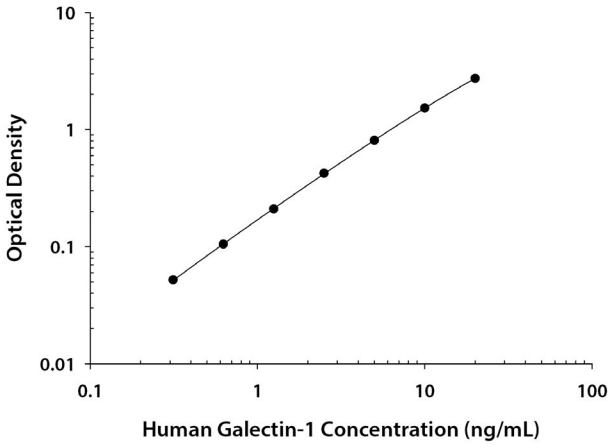
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 Galectin-1 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

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	0.028 0.028	0.028	—
0.313	0.080 0.081	0.080	0.052
0.625	0.133 0.133	0.133	0.105
1.25	0.237 0.240	0.238	0.210
2.5	0.447 0.456	0.451	0.423
5	0.821 0.853	0.837	0.809
10	1.546 1.567	1.556	1.528
20	2.742 2.780	2.761	2.733

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)	1.96	4.66	8.51	2.07	5.02	9.37
Standard deviation	0.139	0.408	0.483	0.197	0.444	0.701
CV (%)	7.1	8.8	5.7	9.5	8.8	7.5

RECOVERY

The recovery of Galectin-1 spiked to levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	93-115%
Serum (n=4)	96	82-104%
EDTA plasma (n=4)	96	87-102%
Heparin plasma (n=4)	93	80-102%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of Galectin-1 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)	Human milk* (n=4)
1:2	Average % of Expected	101	98	103	100	106	107
	Range (%)	97-107	97-99	99-107	95-103	103-108	103-111
1:4	Average % of Expected	94	96	105	99	108	110
	Range (%)	92-95	94-98	100-109	93-102	103-113	104-117
1:8	Average % of Expected	92	97	108	101	102	110
	Range (%)	89-97	93-103	100-115	93-105	88-115	100-117
1:16	Average % of Expected	87	97	106	102	———	107
	Range (%)	83-93	92-106	98-116	93-107	———	104-110

*Samples were diluted prior to assay.

SENSITIVITY

Twenty-nine assays were evaluated and the minimum detectable dose (MDD) of Galectin-1 ranged from 0.008-0.129 ng/mL. The mean MDD was 0.022 ng/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 *E. coli*-expressed recombinant human Galectin-1.

SAMPLE VALUES

Serum/Plasma/Saliva/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of Galectin-1 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)	18.1	13.9-28.2	3.40
EDTA plasma (n=36)	20.6	15.9-30.4	3.63
Heparin plasma (n=36)	19.7	15.2-29.1	3.33
Human milk (n=13)	8.83	2.15-20.8	5.07

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Saliva (n=12)	6.29	92	ND-12.2

ND=Non-detectable

Cell Culture Supernates:

Human peripheral blood leukocytes were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were unstimulated or stimulated with 10 µg/mL of PHA. Aliquots of the cell culture supernates were removed on days 1 and 6 and assayed for levels of Galectin-1.

Condition	Day 1 (ng/mL)	Day 6 (ng/mL)
Unstimulated	13.0	10.6
Stimulated	19.3	34.6

SW480 human colorectal adenocarcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum and incubated at 37 °C with 5% CO_2. An aliquot of the cell culture supernate was removed, assayed for Galectin-1, and measured 6.70 ng/mL.

MDA-MB-231 human breast cancer cells were cultured in DMEM supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for Galectin-1, and measured 99.2 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Galectin-1.

The factors listed below were prepared at 200 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range recombinant human Galectin-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CD2
CD7
CD43
CD45
Galectin-2
Galectin-3
Galectin-3BP/MAC-2BP
Galectin-4
Galectin-7
Galectin-8
Galectin-9
Galectin-10
Galectin-14
Integrin α4β1
Integrin α4β7
Integrin α5β1
LAM-A1

Recombinant mouse:

Galectin-2
Galectin-3
Galectin-3BP/MAC-2BP
Galectin-4
Galectin-7
Galectin-9

Natural proteins:

human Fibronectin

Other factors:

Lactose

Recombinant mouse Galectin-1 cross-reacts approximately 9.1% in this assay.

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