Human Galectin-3 immunoassay kit

Catalogue number: 31690

For the quantitative determination of human galectin-3
concentrations in serum and plasma samples.

This package insert must be read in its entirety before using this product

FOR RESEARCH USE ONLY

Version: 2.0

TABLE OF CONTENT

INTRODUCTION

Galectins are a family of animal lectins with carbohydrate-binding activity and specificity for N-acetyllactosamine (LacNac). [1] Galectin-3 was the unique chimera type lectins. [2] Galectin-3 lacks a signal peptide for classical secretion but presents and active inside and outside the cell. Human galectin-3, 29-36 KDa, exhibits numerous autocrine and paracrine effects and mediates in cell adhesion, cell activation and apoptosis and up- or down-regulation in cancer [3]. In addition, plays an important role in immune response and inflammation. While the cytosolic galectin-3 involves in the cell proliferation, differentiation and survival.

Serum levels of galectin-3 elevated in Behcet's Diseases[4], Thyroid disease[5], Alzheimer's disease[6], cardiovascular disease such as LAA stroke[7] and in serval cancers especially when they are metastatic[8]. Moreover, circulating levels of galectin-3 are higher in obese humans and indicative for a role in insulin resistance in man.[9]

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich ELISA. The immunoplate is pre-coated with a polyclonal antibody specific for human galectin-3. Standards and samples are pipetted into the wells and any human galectin-3 present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase (HRP)-linked polyclonal antibody specific for human galectin-3 is added to the wells. After a final wash step, an HRP substrate solution is added and colour develops in proportion to the amount of human galectin-3 bound initially. The assay is stopped and the optical density of the wells determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured human galectin-3, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This human galectin-3 ELISA kit is designed for quantification of human galectin-3 in serum and plasma samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Micro-titre Strips (96 wells)-Coated with a polyclonal antibody against human galectin-3, sealed.
2. 10×Wash buffer-50 ml.
3. 5×Assay buffer-20 ml.
4. 100×Detection antibody solution-A biotin labelled polyclonal antibody against human galectin-3, 0.12 ml.
5. Human galectin-3 standard-40 ng of native human galectin-3 in a buffered protein base, lyophilised
6. 200×STP-HRP solution- 0.06 ml
7. Substrate solution- 12 ml, ready for use.
8. Stop solution- 12 ml, ready for use.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
2. 96-well plate or manual strip washer.
3. Buffer and reagent reservoirs.
4. Paper towels or absorbent paper.
5. Plate reader capable of reading absorbency at 450 nm.
6. Distilled water or deionized water.

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the human galectin-3 microplate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer.

Prepare 1×Assay buffer by mixing the 5×Assay buffer (20 ml) with 80 ml of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer.

Prepare 1×Wash buffer by mixing the 10×Wash buffer (50 ml) with 450 ml of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution.

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µl of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

D. 1×STP-HRP solution.

Spin down the 200×STP-HRP solution briefly and dilute the desired amount of the 200×STP-HRP solution 1:200 with 1×Assay buffer, 100 µl of the 1×STP-HRP solution is required per well. Prepare only as much 1×STP-HRP solution as needed. Return the 200×STP-HRP solution to 2-8°C immediately after the necessary volume is removed

PREPARATION OF STANDRADS AND SAMPLES

Human galectin-3 standards: Reconstitute the lyophilised standard with 1 ml of 1×Assay buffer to generate a standard stock solution of 40 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions.
Prepare serially diluted standards using 1×Assay buffer as follows:

Standard volume	Volume of 1×Assay buffer	Concentration
40 ng/ml stock	-	40 ng/ml
250 µl of 20 ng/ml	250 µl	20 ng/ml
250 µl of 10 ng/ml	250 µl	10 ng/ml
250 µl of 5 ng/ml	250 µl	5 ng/ml
250 µl of 2.5 ng/ml	250 µl	2.5 ng/ml
250 µl of 1.25 ng/ml	250 µl	1.25 ng/ml
250 µl of 0.625 ng/ml	250 µl	0.625 ng/ml

1×Assay buffer serves as the zero standard (0 ng/ml).

The reconstituted standard stock should be aliquoted and stored at -20ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample preparation

Serum or plasma sample is generally required a 5-fold dilution in the 1×Assay buffer.

ASSAY PROCEDURE

It is recommended that all standards and samples should be assayed in duplicate.

1. Add 100 µl of standard or sample per well, incubate at room temperature for 2 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual Solution in each well. Add 300 µl of 1x Wash buffer to each well and incubate for 1 minute. Discard the 1xWash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µl of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 3 times as in step 2.
5. Add 100 µl of 1×STP-HRP solution to each well, incubate at room temperature for 20 minutes.
6. Wash each well 4 times as described in step 2.
7. Add 100 µl of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
8. Add 100 µl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
9. Measure absorbance of each well at 450 nm immediately.

CALCULATION

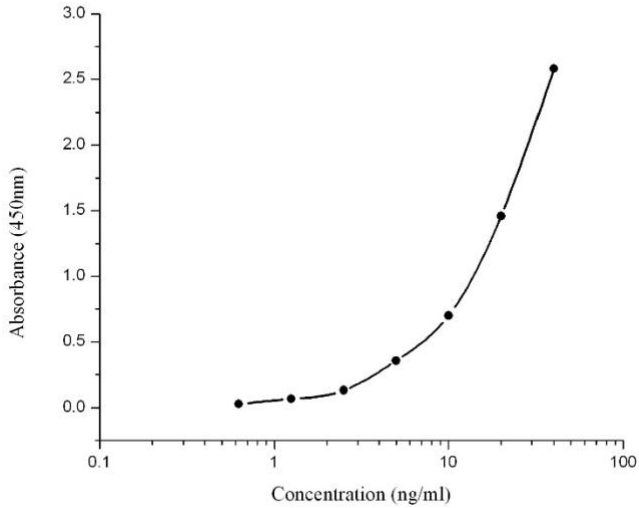
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human galectin-3 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine human galectin-3 concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

Human galectin-3 (ng/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.082	0
0.625	0.111	0.029
1.25	0.15	0.068
2.5	0.213	0.131
5	0.404	0.357
10	0.782	0.70
20	1.542	1.46
40	2.663	2.581

Human galectin-3 standard curve （4 parameters）



ASSAY CHARACTERISTICS

A. Sensitivity:

The lowest level of human galectin-3 that can be measured by this assay is 0.145 ng/ml.

B. Specificity:

Cross reactivity of recombinant proteins:

Analyte	Cross Reactivity
Human FABP4	No
Human LCN2	No
Human Adiponectin	No
Human FGF-21	No

C. Precision:

Intra-assay Precision (Precision within an assay)

Two samples of known concentration were tested 8 times on one plate.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	29.65	1.33	4.5
2	2.26	0.14	6.2

Inter-assay Precision (Precision between assays)

Two samples of known concentration were tested in 8 separate assays.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	29.5	1.64	5.5
2	2.31	0.14	6.1

D. Spike

Serum samples were assayed by adding 90 µl of sample and 10 µl of spike stock solution calculated to yield the intended 0, 2.5, 20 or 40 ng/ml spike concentration

Spike level	Expected (ng/ml)	Observed (ng/ml)	Recovery (%)
Low spike (2.5 ng/ml)	2.23	2.02	90.3
Medium spike (20 ng/ml)	23.1	17.3	74.9
High spike (40 ng/ml)	43.5	32.9	75.6

E. Linearity:

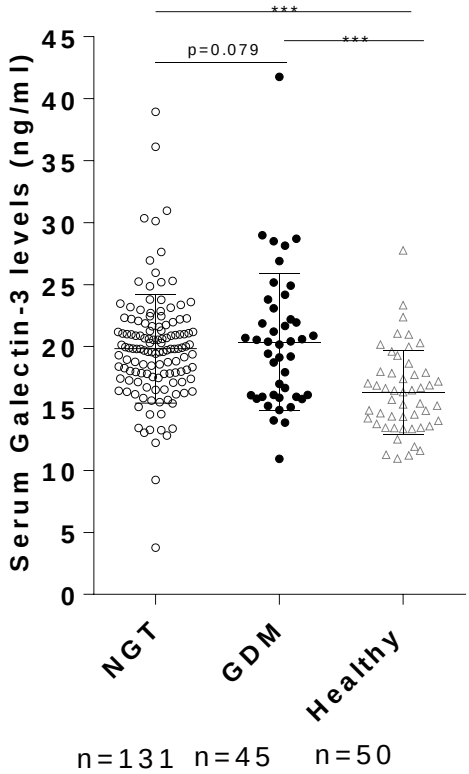
To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human galectin-3 were serially diluted with the 1×Assay buffer to produce samples with values within the dynamic range of the assay.

Sample 1

Dilution	Measured (ng/ml)	Expected (ng/ml)	Recovery (%)
1/2	35.7	40	89.2
1/4	35.5	40	88.6
1/8	36.2	40	90.5

F. Validation:

Serum galectin-3 levels were measured in three different groups, women with normal glucose tolerance during pregnancy (NGT), gestational diabetes mellitus (GDM) and healthy women (age 20-35,Healthy) respectively.



REFERENCE:

1. Dumic, Jerka, Sanja Dabelic, and Mirna Flögel. "Galectin-3: an open-ended story." Biochimica et Biophysica Acta (BBA)-General Subjects 1760.4 (2006): 616-635.
2. Krześlak, Anna, and Anna Lipińska. "Galectin-3 as a multifunctional protein." Cell Mol Biol Lett 9.2 (2004): 305-28.
3. van den Brûle, Frédéric, Stèphane Califice, and Vincent Castronovo. "Expression of galectins in cancer: a critical review." Glycoconjugate journal 19.7 (2002): 537-542.

4. Lee, Y. J., et al. "Serum galectin-3 and galectin-3 binding protein levels in Behçet's disease and their association with disease activity." Clinical and experimental rheumatology 25.4 Suppl 45 (2007): S41-5.
5. Saussez, Sven, et al. "Serum galectin-1 and galectin-3 levels in benign and malignant nodular thyroid disease." Thyroid 18.7 (2008): 705-712.
6.Wang, Xuexin, et al. "Elevated Galectin-3 levels in the serum of patients with Alzheimer's disease." American Journal of Alzheimer's Disease & Other Dementias® 30.8 (2015): 729-732.
7. He, Xin-Wei, et al. "Serum levels of galectin-1, galectin-3, and galectin-9 are associated with large artery atherosclerotic stroke." Scientific reports 7 (2017).
8. Nangia-Makker, Pratima, et al. "Galectin-3 cleavage: a novel surrogate marker for matrix metalloproteinase activity in growing breast cancers." Cancer research 67.24 (2007): 11760-11768.
9. Li, Pingping, et al. "Hematopoietic-derived galectin-3 causes cellular and systemic insulin resistance." Cell 167.4 (2016): 973-984

SUMMARY OF ASSAY PROCEDURE

Add 100 µl of standard or sample to each well.
↓
Incubate at room temperature for 2 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µl of 1xDetection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µl of 1x STP-HRP solution to each well.
↓
Aspirate and wash each well four times.
↓
Add 100 µl of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µl of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation