ImmunoDiagnostics Limited

**FOR RESEARCH USE ONLY**

# Human Galectin-3 ELISA Kit

(Catalog Number: 31690)

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


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## INTRODUCTION

Galectins are a family of animal lectins with carbohydrate-binding activity and specificity for N-acetyllactosamine (LacNac)[1]. Galectin-3 is the unique chimera type lectins[2]. Even though galectin-3 lacks signal peptide, it can be secreted outside the cell and is active both 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, it 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 are elevated in Behcet's disease[4], thyroid disease[5], Alzheimer's disease[6], cardiovascular disease such as LAA stroke[7] and in several 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 enzyme-linked immunosorbent assay (ELISA). The microtiter plate 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 color develops in proportion to the amount of human galectin-3 bound initially. The assay is stopped, and the optical density of the wells is 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. Microtiter 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, lyophilized
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 microtiter plate, 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**

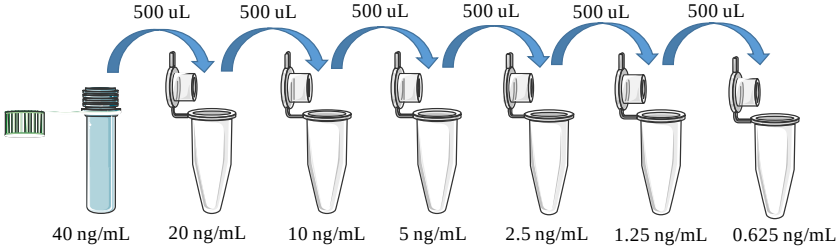
Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 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 STANDARDS AND SAMPLES**

**Human Galectin-3 Standards:** Reconstitute the lyophilized 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. Pipette 500 µL of 1×Assay buffer to 20, 10, 5, 2.5, 1.25, 0.625 ng/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.




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 generally requires a **5-fold** dilution in the 1×Assay buffer. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

## ASSAY PROCEDURE

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

1. Add 100 µL of standard or sample per well, incubate at room temperature for 2 hours.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash 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.

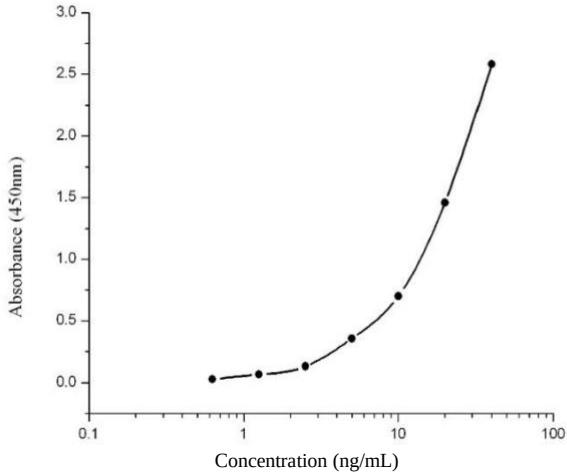
3. Determine human galectin-3 concentration of samples from standard curve and multiply the value by the dilution factor.

**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**

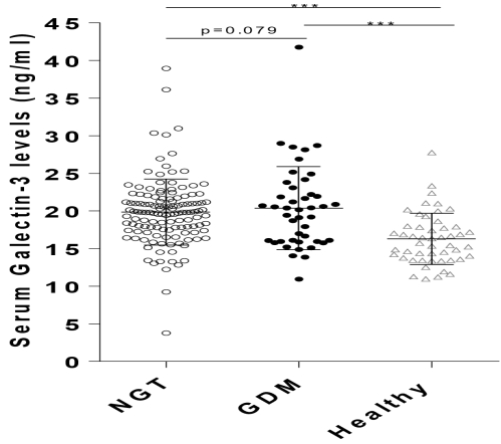
To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human galectin-3 were serially diluted with the 1×Assay buffer to produce samples with values within the dynamic range of the assay.

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;n=131), gestational diabetes mellitus (GDM;n=45) and healthy women (Healthy; n=50; age 20-35) respectively.

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## SUMMARY OF ASSAY PROCEDURE

Add 100 µL of standard or sample to each well.
↓
Incubate at room temperature for 2 hours.
↓
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 1xSTP-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