Human Galectin-3 ELISA

For the quantitative determination of human Galectin-3 in cell culture supernatants, serum, plasma or other body fluids.

Cat. No. KT-022

For research use only, not for use in diagnostic procedures.

Rev. 112006

PRODUCT INFORMATION

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PRODUCT

The ***K-ASSAY®*** Human Galectin-3 ELISA is an enzyme-linked immunosorbent assay for the quantitative determination of human Galectin-3 in cell culture supernatants, serum, plasma or other body fluids. **The Human Galectin-3 ELISA is for research use only. Not for use in diagnostic or therapeutic procedures.**

DESCRIPTION

Galectin-3 is a 26 kDa β-galactoside-binding protein belonging to the Galectin family, which consists of more than ten members. Galectin-3 is composed of a carboxyl-terminal carbohydrate recognition domain (CRD) and amino-terminal tandem repeats. Galectin-3 normally distributes in epithelia of many organs and various inflammatory cells, including macrophages as well as dendritic cells and Kupfer cells. The expression of this lectin is up-regulated during inflammation, cell proliferation and cell differentiation and through transactivation by viral proteins. Its expression is also affected by neoplastic transformation: up-regulation is found in certain types of lymphomas, and thyroid carcinoma, while it is down regulated in other types of malignancies such as colon, breast, ovarian and uterine carcinomas. The expression of Galectin-3 has a strong correlation with the grade and malignant potential of primary brain tumors. Increased Galectin-3 levels have also been noted in human atherosclerotic lesions. These findings suggest that Galectin-3 expression is affected during these physiological and pathological responses. Galectin-3 has been shown to function through both intracellular and extracellular actions. It is a component of heterogeneous nuclear ribonuclear protein (hnRNP), a factor in pre-mRNA splicing and has been found to control cell cycle and prevent T-cell apoptosis through interaction with the Bcl-2 family members. On the other hand, this protein, which is secreted from monocytes/macrophages and epithelial cells has been demonstrated to function as an extracellular molecule in activating various types of cells such as monocytes/ macrophages, mast cells, neutrophils and lymphocytes. Galectin-3 has been shown to mediate cell-cell and cell-extracellular matrix interactions and acts as a novel chemoattractant for monocytes and macrophages.

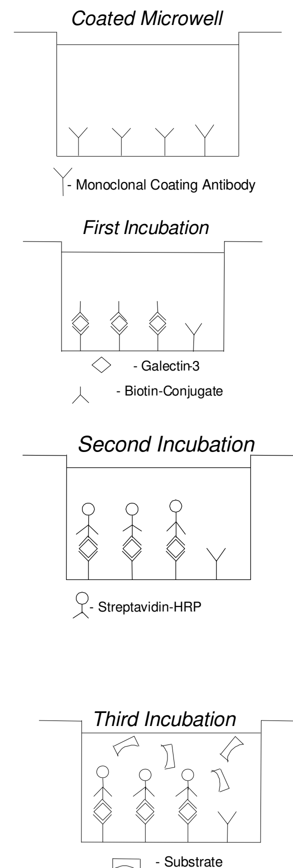
PRINCIPLE

An anti-human Galectin-3 monoclonal coating antibody is adsorbed onto microwells.

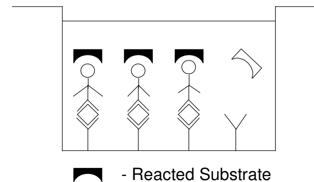
Human Galectin-3 present in the sample or calibrator binds to antibodies adsorbed to the microwells; a biotin-conjugated anti-human Galectin-3 antibody is added and binds to human Galectin-3 captured by the first antibody.

Following incubation, unbound biotin-conjugated anti-human Galectin-3 is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human Galectin-3 antibody.

Following incubation, unbound streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.



A colored product is formed in proportion to the amount of human Galectin-3 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A calibration curve is prepared from seven human Galectin-3 calibrator dilutions and the human Galectin-3 sample concentration is determined.



COMPONENTS

1 aluminum pouch with a **Microwell Plate coated with Monoclonal Antibody** (mouse) to human Galectin-3

1 vial (100 µL) **Biotin-Conjugate** anti-human Galectin-3 polyclonal antibody (rabbit)

1 vial (150 µL) **Streptavidin-HRP Concentrate**

2 vials human **Galectin-3 Calibrator,** lyophilized, 50 ng/mL upon reconstitution

1 vial (5 mL) **Assay Buffer Concentrate** 20X (Phosphate-Buffered Saline with 1% Tween 20 and protein stabilizer)

1 bottle (50 mL) **Wash Buffer Concentrate** 20X (Phosphate-Buffered Saline with 1% Tween 20)

1 bottle (12 mL) **Sample Diluent**

1 vial (15 mL) **Substrate Solution** (tetramethyl-benzidine)

1 vial (12 mL) **Stop Solution** (1M Phosphoric acid)

1 vial (0.4 mL) **Blue Dye**

1 vial (0.4 mL) **Red Dye**

1 vial (0.4 mL) **Green Dye**

4 adhesive **Plate Seals**

MATERIALS REQUIRED BUT NOT PROVIDED

- 5 mL and 10 mL graduated pipettes.
- 5 µL to 1,000 µL adjustable single channel micropipettes with disposable tips.
- 50 µL to 300 µL adjustable multi-channel micropipette with disposable tips.
- Multi-channel micropipette reservoir.
- Beakers, flasks, cylinders necessary for preparation of reagents.
- Device for delivery of wash solution (multi-channel wash bottle or automatic wash system).
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wavelength).
- Glass-distilled or de-ionized water.
- Statistical calculator with program to perform linear regression analysis.

STORAGE

Store kit reagents at 4°C. Immediately after use remaining reagents should be returned to cold storage (4°C). Expiration date of the kit and reagents is stated on labels.

The expiration date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

SPECIMEN COLLECTION

Cell culture supernatants, human serum, plasma (EDTA) or other biological samples will be suitable for use in the assay. Remove the serum or plasma from the clot or red cells, respectively, as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive Galectin-3. Addition of protease inhibitors may account for better stability of samples. If samples are to be run within 24 hours, they may be stored at 4°C. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

For stability of samples refer to page 11.

Pay attention to a possible “Hook Effect” due to high sample concentrations.

PRECAUTIONS FOR USE

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product be handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagents.
- Exposure to acids will inactivate the conjugate.
- Glass-distilled water or de-ionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

PROCEDURES

PREPARATION OF REAGENTS

Buffer Concentrates should be brought to room temperature and should be diluted before starting the test procedure. If crystals have formed in the Buffer Concentrates, warm them gently until they have completely dissolved.

A. Wash Buffer

Pour the entire contents (50 mL) of the **Wash Buffer Concentrate** into a clean 1,000 mL graduated cylinder. Bring the final volume to 1,000 mL with glass-distilled or de-ionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days. Wash Buffer may be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	25	475
1 - 12	50	950

B. Assay Buffer

Mix the contents of the bottle well. Add the contents of the **Assay Buffer Concentrate** (5.0 mL) to 95 mL distilled or de-ionized water and mix gently to avoid foaming. Store at 4°C. Please note that the Assay Buffer is stable for 30 days. Assay Buffer may be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. Preparation of Biotin-Conjugate

Please note that the Biotin-Conjugate should be used within 30 minutes after dilution.
The Biotin-Conjugate must be diluted 1:100 with **Assay Buffer** just prior to use in a clean plastic test tube according to the following table:

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. Preparation of human Galectin-3 Calibrator

Reconstitute the human **Galectin-3 Calibrator** by addition of the volume of distilled water as stated on vial label. Mix or swirl gently to insure complete and homogeneous solubilization. (Concentration of reconstituted calibrator = 50 ng/mL)

E. Preparation of Streptavidin-HRP

Please note that the Streptavidin-HRP should be used within 30 minutes after dilution.
Make a 1:400 dilution of the **Streptavidin-HRP Concentrate** with Assay Buffer in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (mL)
1 - 6	0.015	5.985
1 - 12	0.030	11.970

F. **Addition of Color Dyes** (optional step)

In order to help our customers to avoid any mistakes in pipetting, KAMIYA BIOMEDICAL COMPANY now offers a new tool that helps to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colors to each step of the ELISA procedure.

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted.

Alternatively, the dye solutions from the stocks provided (**Blue Dye, Green Dye, Red Dye**) can be added to the reagents according to the following guidelines:

1. Sample Diluent: Before sample dilution, add the **Blue Dye** at a dilution of 1:250 (see table below) to the Sample Diluent according to the test protocol. After addition of **Blue Dye**, proceed according to the package insert.

5 mL Sample Diluent	20 µL **Blue Dye**
12 mL Sample Diluent	48 µL **Blue Dye**
50 mL Sample Diluent	200 µL **Blue Dye**

2. Biotin-Conjugate: Before dilution of the concentrated biotin-conjugate, add the **Green Dye** at a dilution of 1:100 (see table below) to the Assay Buffer used for the final biotin-conjugate dilution. Proceed after addition of **Green Dye** according to the package insert, preparation of the biotin-conjugate.

3 mL Assay Buffer	30 µL **Green Dye**
6 mL Assay Buffer	60 µL **Green Dye**

3. Streptavidin-HRP: Before dilution of the concentrated Streptavidin-HRP, add the **Red Dye** at a dilution of 1:250 (see table below) to the Assay Buffer used for the final Streptavidin-HRP dilution. Proceed after addition of **Red Dye** according to the package insert, preparation of Streptavidin-HRP.

6 mL Assay Buffer	24 µL **Red Dye**
12 mL Assay Buffer	48 µL **Red Dye**

PROTOCOLS

a. Prepare reagents immediately before use and mix them thoroughly without foaming.

b. Determine the number of Microwell Strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and calibrators. Each sample, calibrator, blank and optional control sample should be assayed in duplicate. Remove extra **Microwell Strips coated with Monoclonal Antibody** (mouse) to human Galectin-3 from holder and store in foil bag with the desiccant provided at 4°C sealed tightly.

c. Wash the microwell strips twice with approximately 400 µL **Wash Buffer** per well with thorough aspiration of microwell contents between washes. Allow Wash Buffer to sit in wells for about 10-15 seconds before aspiration.Take care not to scratch the surface of the microwells.

   After the last wash, tap microwell strips on an absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

d. Add 100 µL of **Sample Diluent** in duplicate to all calibrator wells. Prepare calibrator dilutions by pipetting 100 µL of reconstituted human **Galectin-3 Calibrator** (Refer to preparation of reagents), in duplicate, into wells A1 and A2. Mix the contents of wells A1 and A2 by repeated aspiration and ejection, and transfer 100 µL to wells B1 and B2, respectively. Take care not to scratch the inner surface of the microwells. Continue this procedure five times, creating two rows of human Galectin-3 calibrator dilutions ranging from 25 to 0.39 ng/mL. Discard 100 µL of the contents from the last microwells (G1, G2) used.

Figure 1. Preparation of human Galectin-3 calibrator dilutions:

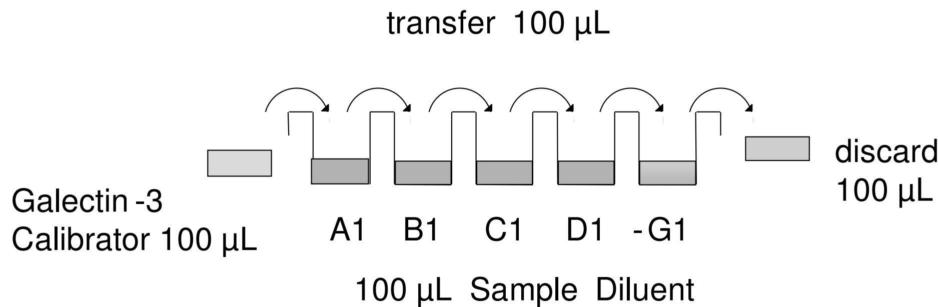


Figure 2. Diagram depicting an example of the arrangement of blanks, calibrators and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Calibrator 1 (25.00 ng/mL)	Calibrator 1 (25.00 ng/mL)	Sample 1	Sample 1
B	Calibrator 2 (12.50 ng/mL)	Calibrator 2 (12.50 ng/mL)	Sample 2	Sample 2
C	Calibrator 3 (6.25 ng/mL)	Calibrator 3 (6.25 ng/mL)	Sample 3	Sample 3
D	Calibrator 4 (3.13 ng/mL)	Calibrator 4 (3.13 ng/mL)	Sample 4	Sample 4
E	Calibrator 5 (1.56 ng/mL)	Calibrator 5 (1.56 ng/mL)	Sample 5	Sample 5
F	Calibrator 6 (0.78 ng/mL)	Calibrator 6 (0.78 ng/mL)	Sample 6	Sample 6
G	Calibrator 7 (0.39 ng/mL)	Calibrator 7 (0.39 ng/mL)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

e. Add 100 µL of **Sample Diluent**, in duplicate, to the blank wells.

f. Add 50 µL of **Sample Diluent** to the sample wells.

g. Add 50 µL of each **Sample**, in duplicate, to the designated wells.

h. Prepare **Biotin-Conjugate** (Refer to preparation of reagents).

i. Add 50 µL of diluted **Biotin-Conjugate** to all wells, including the blank wells.

j. Cover with a **Plate Seal** and incubate at room temperature (18° to 25°C) for 2 hours on a microplate shaker set at 200 rpm.

k. Remove **Plate Seal** and empty wells. Wash microwell strips 3 times according to point c of the test protocol. Proceed immediately to the next step.

l. Prepare **Streptavidin-HRP** (Refer to preparation of reagents).

m. Add 100 µL of diluted **Streptavidin-HRP** to all wells, including the blank wells.

n. Cover with a **Plate Seal** and incubate at room temperature (18° to 25°C) for 1 hour on a microplate shaker at 200 rpm, if available.

o. Remove **Plate Seal** and empty wells. Wash microwell strips 3 times according to point c of the test protocol. Proceed immediately to the next step.

p. Pipette 100 µL of **TMB Substrate Solution** to all wells, including the blank wells.

q. Incubate the microwell strips at room temperature (18° to 25°C) for about **10 minutes**. Avoid direct exposure to intense light. **The color development on the plate should be monitored and the substrate reaction stopped (see point r. of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.**
It is recommended to add the stop solution when the highest calibrator has reached a dark blue color. The color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Calibrator 1 has reached an O.D. of 0.6-0.65.

r. Stop the enzyme reaction by quickly pipetting 100 µL of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 4°C in the dark.

s. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wavelength (optional 620 nm as the reference wavelength; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human Galectin-3 calibrators.

Note: In case of incubation without shaking, the obtained O.D. values may be lower than indicated below. Nevertheless the results are still valid.

CALCULATIONS

- Calculate the average absorbance values for each set of duplicate calibrators and samples. Duplicates should be within 20% of the mean.

- Create a calibration curve by plotting the mean absorbance for each calibrator concentration on the ordinate against the human Galectin-3 concentration on the abscissa. Draw a best-fit curve through the points of the graph (a 5-parameter curve fit is recommended).

- To determine the concentration of circulating human Galectin-3 for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the calibration curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human Galectin-3 concentration.

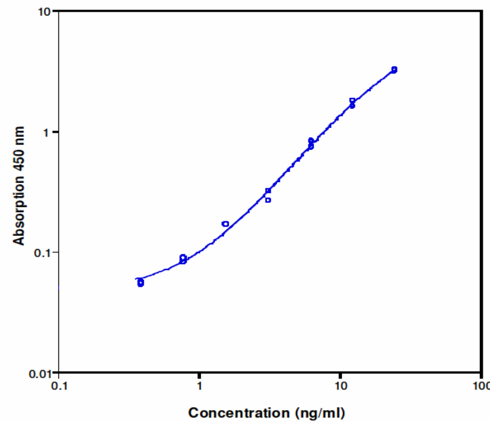
- **For samples which have been diluted according to the instructions given in this manual (1:2) the concentration read from the calibration curve must be multiplied by the dilution factor (x2).**

  Note: Calculation of samples with a concentration exceeding calibrator 1 may result in incorrect, low human Galectin-3 levels (Hook Effect). Such samples require further external predilution according to expected Galectin-3 values with Sample Diluent in order to precisely quantitate the actual human Galectin-3 level.

- It is suggested that each testing facility establishes a control sample of known human Galectin-3 concentration and runs this additional control with each assay. If the values obtained are not within the expected range of this control, the assay results may be invalid.

- A representative calibration curve is shown in Figure 3. This curve cannot be used to derive test results. Every laboratory must prepare a calibration curve for each group of microwell strips assayed.

Figure 3: Representative calibration curve for Human Galectin-3 ELISA. Human Galectin-3 was diluted in serial two-fold steps in Sample Diluent. Do not use this calibration curve to derive test results. A calibration curve must be run for each group of microwell strips assayed.



Typical data using the Human Galectin-3 ELISA

Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Calibrator	human Galectin-3 Concentration (ng/mL)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	25.00	3.214	3.195	0.6
	25.00	3.176		
2	12.50	1.779	1.681	5.7
	12.50	1.585		
3	6.25	0.818	0.777	5.3
	6.25	0.736		
4	3.13	0.313	0.287	8.8
	3.13	0.262		
5	1.56	0.168	0.169	0.2
	1.56	0.169		
6	0.78	0.088	0.085	3.4
	0.78	0.082		
7	0.39	0.056	0.055	1.5
	0.39	0.054		
Blank	0	0.028	0.025	12.6
	0	0.022		

The OD values of the calibration curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore, shelf-life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

LIMITATIONS

- Since exact conditions may vary from assay to assay, a calibration curve must be established for every run.

- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.

- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

- Improper or insufficient washing at any stage of the procedure can result in either false positive or false negative results. Completely empty wells before dispensing fresh Wash Buffer, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibody (HAMA). HAMA may interfere with assays utilizing mouse monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to mouse immunoglobulins can still be analyzed in such assays when mouse immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

PERFORMANCE CHARACTERISTICS

A. Sensitivity
The limit of detection of human Galectin-3 is defined as the analyte concentration resulting in an absorption reading significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be 0.12 ng/mL (mean of 6 independent assays).

B. Reproducibility

a. Intra-assay
Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human Galectin-3. Two calibration curves were run on each plate.

Data below show the mean human Galectin-3 concentration and the coefficient of variation for each sample. The overall Intra-assay coefficient of variation has been calculated to be 6.4%.

Positive Sample	Experiment	Mean human Galectin-3 Conc. (ng/mL)	CV (%)
1	1	12.38	14
	2	15.61	2
	3	11.81	5
2	1	1.88	10
	2	2.36	3
	3	2.60	5
3	1	1.52	5
	2	1.77	5
	3	1.49	6
4	1	17.23	8
	2	15.65	2
	3	16.89	3
5	1	1.74	13
	2	1.68	4
	3	1.65	10
6	1	3.87	9
	2	5.48	6
	3	5.59	5

b. Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 6 serum samples containing different concentrations of human Galectin-3. Two calibration curves were run on each plate. Data below show the mean human Galectin-3 concentration and the coefficient of variation calculated on 18 determinations of each sample. The overall inter-assay coefficient of variation has been calculated to be 11.4%.

Sample	Mean human Galectin-3 Conc. (ng/mL)	CV (%)
1	13.27	15.5
2	2.28	16.0
3	1.59	9.7
4	16.59	5.0
5	1.69	3.0
6	4.98	19.4

C. Spike Recovery

The spike recovery was evaluated by spiking four levels of human Galectin-3 into pooled normal human serum. Recoveries were determined in three independent experiments with 6 replicates each. The unspiked serum was used as blank in these experiments. Recoveries ranged from 73% to 88% with an overall mean recovery of 77%.

D. Dilution Parallelism

Three serum samples with different levels of human Galectin-3 were assayed at serial two-fold dilutions (1:2, 1:4, 1:8 and 1:16) with 4 replicates each. Recoveries ranged from 94% to 128% with an overall mean recovery of 115%.

		human Galectin-3 Concentration (ng/mL)		
Sample	Dilution	Expected Value	Observed Value	% Recovery of Exp. Value
1	1:2	--	13.07	--
	1:4	6.54	8.35	127.7
	1:8	4.17	4.72	113.1
	1:16	2.36	2.66	112.8
2	1:2	--	4.10	--
	1:4	2.05	2.53	123.4
	1:8	1.27	1.19	93.8
	1:16	0.59	0.63	106.2
3	1:2	--	6.32	--
	1:4	3.16	4.05	128.2
	1:8	2.02	2.46	121.4
	1:16	1.23	1.36	111.0

E. Sample Stability

a. Freeze-Thaw Stability

Aliquots of spiked and unspiked serum samples were stored frozen at –20°C and thawed up to 5 times, and the human Galectin-3 levels determined. There was no significant loss of human Galectin-3 immunoreactivity by freezing and thawing up to 5 times.

b. Storage Stability

Aliquots of spiked and unspiked serum samples were stored at -20°C, 4°C, room temperature (RT) and at 37°C, and the human Galectin-3 level was determined after 24 hours. There was no significant loss of human Galectin-3 immunoreactivity during storage under the above conditions.

F. Specificity

The interference of circulating factors of the immune system was evaluated by spiking these proteins at physiologically relevant concentrations into a Galectin-3 positive serum. There was no detectable cross reactivity.

REAGENT PREPARATION SUMMARY

A. Wash Buffer

Add **Wash Buffer Concentrate** 20X (50 mL) to 950 mL distilled water.

B. Assay Buffer

Number of Strips	Assay Buffer Concentrate (mL)	Distilled Water (mL)
1 - 6	2.5	47.5
1 - 12	5.0	95.0

C. Biotin-Conjugate

Make a 1:100 dilution according to the table.

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (mL)
1 - 6	0.03	2.97
1 - 12	0.06	5.94

D. Calibrator

Reconstitute human Galectin-3 Calibrator by addition of distilled water as stated on vial label.

E. Streptavidin-HRP

Make a 1:400 dilution according to the table.

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (mL)
1 - 6	0.015	5.985
1 - 12	0.030	11.970

TEST PROTOCOL SUMMARY

- Wash microwell strips twice with **Wash Buffer.**
- Add 100 µL **Sample Diluent**, in duplicate, to all calibrator wells.
- Pipette 100 µL reconstituted human **Galectin-3 Calibrator** into the first wells and create calibrator dilutions ranging from 25.00 to 0.39 ng/mL by transferring 100 µL from well to well. Discard 100 µL from the last well.
- Add 100 µL **Sample Diluent**, in duplicate, to the blank wells.
- Add 50 µL **Sample Diluent** to the sample wells.
- Add 50 µL **Sample**, in duplicate, to designated wells.
- Prepare **Biotin-Conjugate.**
- Add 50 µL of diluted **Biotin-Conjugate** to all wells.
- Cover microwell strips and incubate 2 hours at room temperature (18° to 25°C).
- Prepare **Streptavidin-HRP.**
- Empty and wash microwell strips 3 times with **Wash Buffer.**
- Add 100 µL of diluted **Streptavidin-HRP** to all wells.
- Cover microwell strips and incubate 1 hour at room temperature (18° to 25°C).
- Empty and wash microwell strips 3 times with **Wash Buffer.**
- Add 100 µL of **TMB Substrate Solution** to all wells including blank wells.
- Incubate the microwell strips for 10 minutes at room temperature (18° to 25°C).

- Add 100 µL **Stop Solution** to all wells including blank wells.
- Blank microwell reader and measure color intensity at 450 nm.

Note: **For samples which have been diluted according to the instructions given in this manual (1:2) the concentration read from the calibration curve must be multiplied by the dilution factor (x2).**

<u>FOR RESEARCH USE ONLY</u>
<u>NOT FOR USE IN DIAGNOSTIC PROCEDURES</u>