

Human LGALS9/Galectin-9 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00175

Size: 5*96T

Sensitivity: 3.9 pg/mL

Range: 31.25-2000 pg/mL, 62.5-4000 pg/mL

Usage: For the quantitative detection of human LGALS9/Galectin-9 concentrations in serum, plasma, urine and human milk.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

Table of content	page
1. Background	3
2. Principle	3
3. Required Materials	3
4. Kit Components and Storage	4
5. Safety Notes	4
6. Sample Collection and Storage	4
7. Regent Preparation	5
8. Assay Procedure Summary	7
9. Validation Data	8
9.1 Standard curve	8
9.2 Precision	9
9.3 Recovery	10
9.4 Sample values	10
9.5 Sensitivity	10
9.6 Linearity	11
10. References	11

1. Background

The galectins, formerly known as S-type lectins, are a family of β -galactoside-binding proteins implicated in modulating cell-cell and cell-matrix interactions. Galectin-9 (LGALS9) is a tandem repeat-type member of the galectin family. It has three isoforms (named galectin-9L, galectin-9M, and galectin-9S): long type of 355 amino acids, medium type of 323 amino acids, and short type of 311 amino acids. Galectin-9 is ubiquitously expressed in a variety of tissues, including lymph nodes and spleen, and overexpressed in Hodgkin disease tissue. It is involved in chemoattraction, apoptosis, and the regulation of cell differentiation and has anti-allergic effects. It has been reported that galectin-9 is a ligand for Tim-3, through which galectin-9 can induce T-helper type 1 lymphocyte (Th1) death.

2. Principle



Sandwich ELISA structure (Detection antibody labeled with biotin)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with biotin also binds to the analyte. Streptavidin-HRP binds to the biotin. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

- 3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.
- 3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.
- 3.3 Plate washer: automated or manual.
- 3.4 Absorbent paper towels.
- 3.5 Glass or plastic tubes to prepare standard and sample dilutions.
- 3.6 Beakers and graduated cylinders.
- 3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	5 plates	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 4000 pg/bottle; lyophilized	10 bottles	
Detection antibody, biotinylated (100×) - 600 μ L/vial*	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100×) - 600 μ L/vial*	1 vial	
Sample Diluent PT 1-eg - 150 mL/bottle. For human serum	1 bottle	
Sample Diluent PT 3-eg - 150 mL/bottle. For human plasma	1 bottle	
Sample Diluent PT 3 - 150 mL/bottle. For human milk	1 bottle	
Sample Diluent PT 4 - 150 mL/bottle. For urine	1 bottle	
Detection Diluent - 150 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 150 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 60 mL/bottle	1 bottle	
Stop Solution - 60 mL/bottle	1 bottle	
Plate Cover Seals	15 pieces	

* Centrifugation immediately before use

5. Safety Notes

- 5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
- 5.2 Do not use the kit after the expiration date.
- 5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.
- 5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.
- 5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

- 6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.
- 6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.
- 6.3 Urine: Collect urine samples and centrifuge for 20 minutes at 1000xg. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
- 6.4 Human Milk: Collect milk samples and Centrifuge for 15 minutes at 1000xg at 2-8°C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately.

7. Reagent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Detection Antibody (1X): Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μ L 100X Detection Antibody + 990 μ L Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).

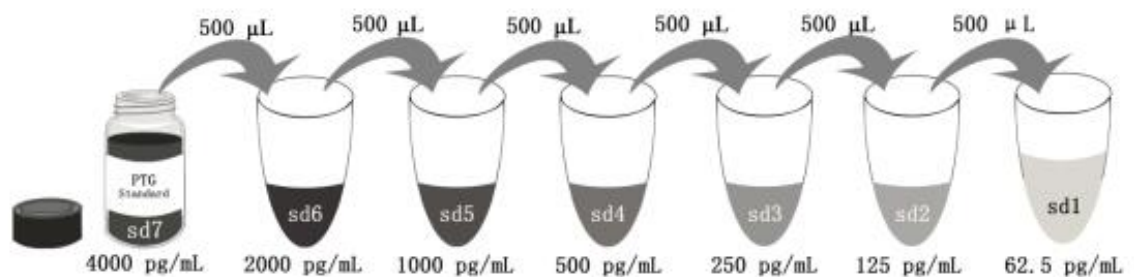
7.3 Streptavidin-HRP (1X): Dilute 100X Streptavidin-HRP 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μ L 100X Streptavidin-HRP + 990 μ L Detection Diluent (Centrifuge the 100X Streptavidin-HRP solution for a few seconds prior to use).

7.4 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:4 is recommended for human serum and plasma; 1:80 or 1:160 is recommended for human milk and urine.

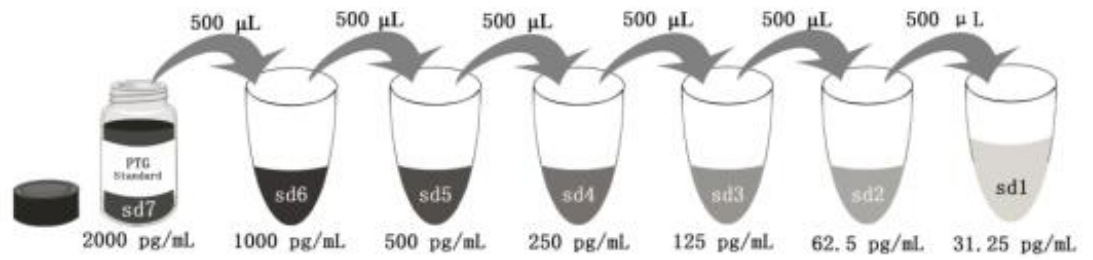
7.5 Standard Serial Dilution:

For human serum sample, add 1 mL Sample Diluent PT 1-eg in standard; For human plasma sample, add 1 mL Sample Diluent PT 3-eg in standard.



Add # μ L of Standard diluted in the previous step	—	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L
# μ L of Sample Diluent PT 1-eg or PT 3-eg	1000 μ L	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L	500 μ L
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

For human milk sample, add 2 mL Sample Diluent PT 3 in standard; For urine sample, add 2 mL Sample Diluent PT 4 in standard.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 3 or PT 4	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and Streptavidin-HRP can be used immediately). 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.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group, standard group and sample group, add 100 μ L of each standard and sample to the appropriate wells. (Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, it is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 μ L per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 μ L of 1X Detection Antibody solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 μ L of 1X Streptavidin-HRP solution (refer to Reagent Preparation 7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C.

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 μ L of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 μ L of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

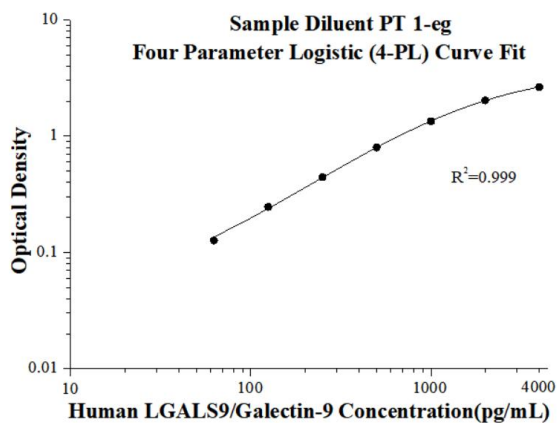
8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve-fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 μ L	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 μ L	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 μ L	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 μ L	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 μ L	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

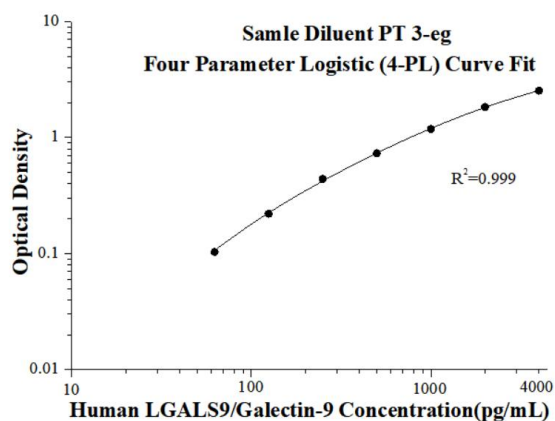
9. Validation Data

9.1 Standard curve

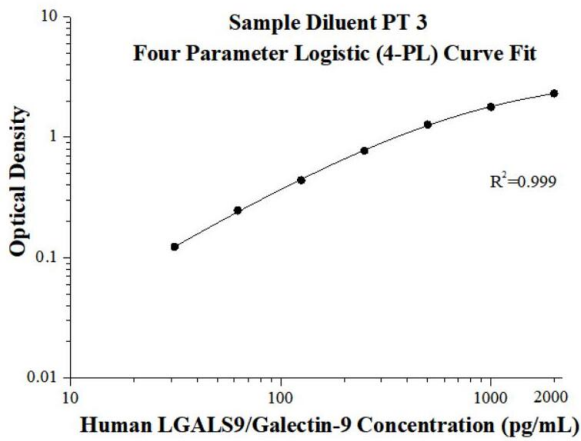
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



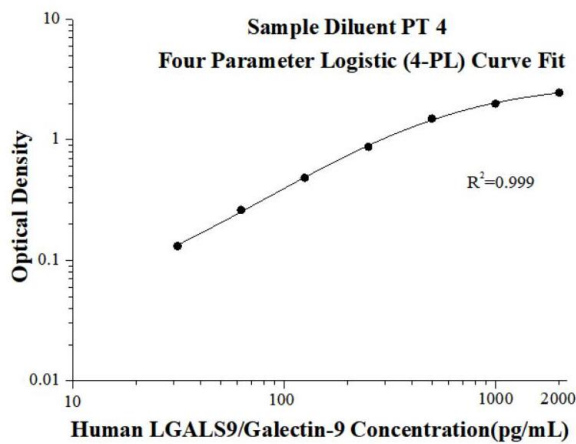
(pg/mL)	O.D	Average	Corrected
0	0.023 0.021	0.022	-
62.5	0.149 0.148	0.149	0.127
125	0.261 0.275	0.268	0.246
250	0.506 0.426	0.466	0.444
500	0.844 0.794	0.819	0.797
1000	1.379 1.339	1.359	1.337
2000	2.099 1.999	2.049	2.027
4000	2.688 2.629	2.659	2.637



(pg/mL)	O.D	Average	Corrected
0	0.020 0.024	0.022	-
62.5	0.126 0.124	0.125	0.103
125	0.262 0.223	0.243	0.221
250	0.430 0.491	0.461	0.439
500	0.761 0.744	0.753	0.731
1000	1.127 1.276	1.202	1.180
2000	1.866 1.833	1.850	1.828
4000	2.479 2.621	2.550	2.528



(pg/mL)	O.D	Average	Corrected
0	0.024 0.024	0.024	-
31.25	0.142 0.152	0.147	0.123
62.5	0.270 0.271	0.271	0.247
125	0.511 0.415	0.463	0.439
250	0.818 0.772	0.795	0.771
500	1.279 1.308	1.294	1.270
1000	1.822 1.781	1.802	1.778
2000	2.319 2.342	2.331	2.307



(pg/mL)	O.D	Average	Corrected
0	0.024 0.023	0.024	-
31.25	0.158 0.151	0.155	0.131
62.5	0.294 0.276	0.285	0.262
125	0.475 0.537	0.506	0.483
250	0.864 0.929	0.897	0.873
500	1.471 1.563	1.517	1.494
1000	2.018 2.007	2.013	1.989
2000	2.481 2.475	2.478	2.455

9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	105.2	2.9	2.8
2	20	258.1	10.9	4.2
3	20	887.9	73.7	8.3

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	113.3	4.6	4.1
2	24	291.9	12.9	4.4
3	24	1,078.3	64.0	5.9

9.3 Recovery

The recovery of human LGALS9/Galectin-9 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:16	96	83-101
	1:32	96	85-108
Human plasma	1:16	94	78-127
	1:32	98	79-125
Human milk	1:160	94	74-124
	1:320	93	80-108
Urine	1:160	90	73-105
	1:320	100	93-108

9.4 Sample values

Human serum and plasma samples from healthy volunteers were evaluated for human LGALS9/Galectin-9 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Human serum (n=16)	7,377	3,263-20,868
Human plasma (n=16)	13,487	2,907-35,041
Human milk (n=7)	29,953	8,920-65,337
Urine (n=9)	9,584	743-17,829

9.5 Sensitivity

The minimum detectable dose of human LGALS9/Galectin-9 is 3.9 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, human serum, plasma, human milk and urine were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

Sample Type		Average% of Expected	Range (%)
Human serum (Sample Diluent PT 1-eg)	1:4	102	92-118
	1:8	100	-
	1:16	108	101-116
	1:32	115	109-120
Human plasma (Sample Diluent PT 3-eg)	1:4	100	-
	1:8	90	85-98
	1:16	91	79-107
	1:32	95	88-106
Human milk (Sample Diluent PT 3)	1:80	100	-
	1:160	96	95-97
	1:320	99	90-106
	1:640	93	89-100
Urine (Sample Diluent PT 4)	1:80	100	-
	1:160	106	94-113
	1:320	109	98-117
	1:640	106	95-116

10. References

1. Barondes SH, et al. Galectins: a family of animal beta-galactoside-binding lectins. *Cell*. 25;76(4):597-8 (1994).
2. O Türeci, et al. Molecular definition of a novel human galectin which is immunogenic in patients with Hodgkin's disease. *J Biol Chem*. 272(10):6416-22 (1997).
3. Zhang F, et al. Different roles of galectin-9 isoforms in modulating E-selectin expression and adhesion function in LoVo colon carcinoma cells. *Mol Biol Rep*. 36(5):823-30 (2009).
4. Zhu C, et al. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. *Nat Immunol*. 6(12):1245-52 (2005).