

## Human LGALS9/Galectin-9 Sandwich ELISA Kit Datasheet

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

### General Information

Catalogue Number	KE00175
Product Name	Human LGALS9/Galectin-9 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-2000 pg/mL, 62.5-4000 pg/mL
Tested applications	Quantification ELISA

### Database Links

Entrez Gene	3965
SwissProt	O00182

### Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	<b>Unopened Kit:</b> Store at 2-8°C for 6 months or -20°C for 12 months.  <b>Opened Kit:</b> All reagents stored at 2-8°C for 7 days.  <b>Please use a new standard for each assay.</b>
Protein standard - 4000 pg/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 1-eg - 30 mL/bottle. For serum	1 bottle	
Sample Diluent PT 3-eg - 30 mL/bottle. For plasma	1 bottle	
Sample Diluent PT 3 - 30 mL/bottle. For human milk	1 bottle	
Sample Diluent PT 4 - 30 mL/bottle. For urine	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

**NB: Do not use the kit after the expiration date.**

Sample Diluent PT 1-eg is for protein standard and serum.

Sample Diluent PT 3-eg is for protein standard and plasma.

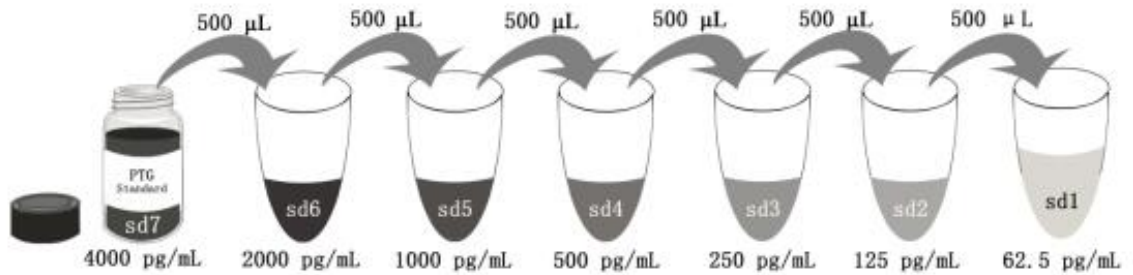
Sample Diluent PT 3 is for protein standard and human milk.

Sample Diluent PT 4 is for protein standard and urine.

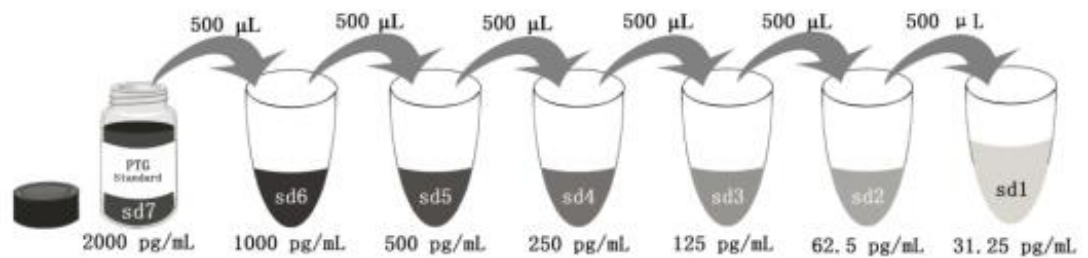
Detection Diluent is for Detection antibody and Streptavidin-HRP.

\*Add 1 mL Sample Diluent PT 1-eg or PT 3-eg in standard. This reconstitution gives a stock solution of 4000 pg/mL.

\*Add 2 mL Sample Diluent PT 3 or PT 4 in standard. This reconstitution gives a stock solution of 2000 pg/mL.



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 1-eg or PT 3-eg	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"



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"

## Product Description

KE00175 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The LGALS9/Galectin-9 ELISA kit is to be used to detect and quantify protein levels of endogenous LGALS9/Galectin-9. The assay recognizes human LGALS9/Galectin-9. An antibody specific for LGALS9/Galectin-9 has been pre-coated onto the microwells. The LGALS9/Galectin-9 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for LGALS9/Galectin-9 is added to detect the captured LGALS9/Galectin-9 protein. For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

## Background

The galectins, formerly known as S-type lectins, are a family of  $\beta$ -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.

## Sample Preparation

The samples may require proper dilution to fall within the range of the assay. A minimum 1:4 dilution is recommended for serum or plasma. A minimum 1:80 or 1:160 dilution is recommended for human milk and urine.

## Safety Notes

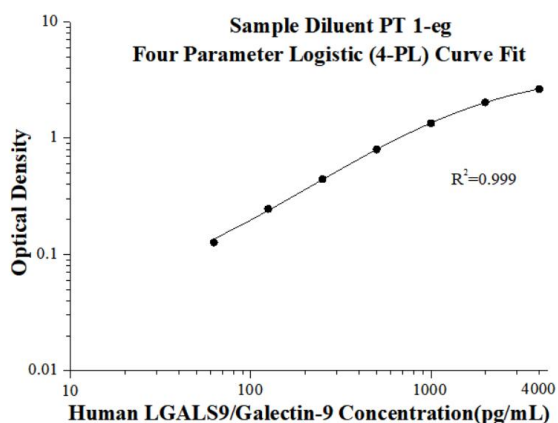
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Assay Procedure Summary

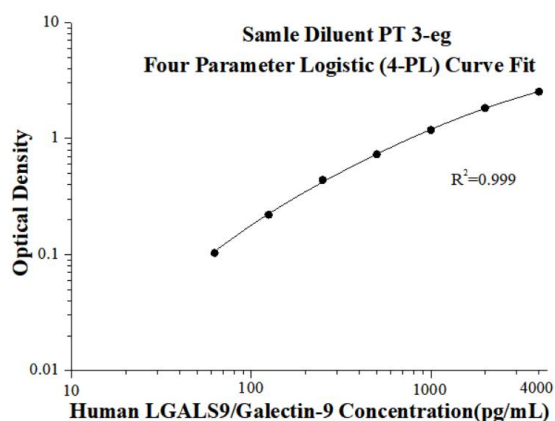
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 $\mu$ L	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 $\mu$ L	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 $\mu$ L	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 $\mu$ L	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 $\mu$ 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.				

## Example data

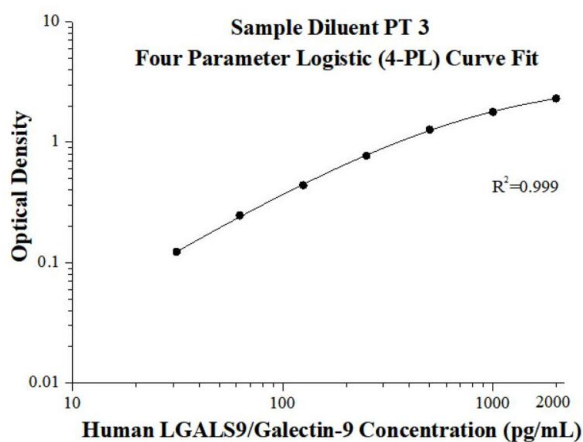
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



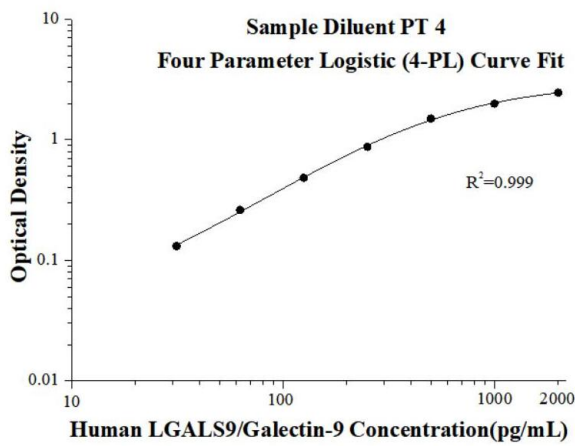
(pg/mL)	O.D	Average	Corrected
0	0.023 0.021	0.022	-
62.5	0.149 0.148	0.149	0.127
125	0.261 0.275	0.268	0.246
250	0.506 0.426	0.466	0.444
500	0.844 0.794	0.819	0.797
1000	1.379 1.339	1.359	1.337
2000	2.099 1.999	2.049	2.027
4000	2.688 2.629	2.659	2.637



(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

## 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

## Recovery

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

Sample Type		Average% of Expected	Range (%)
Serum	1:16	96	83-101
	1:32	96	85-108
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

## Sample Values

Serum and plasma samples from healthy volunteers were evaluated for 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)
Serum (n=16)	7,377	3,263-20,868
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

## Sensitivity

The minimum detectable dose of 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.

## Linearity

To assess the linearity of the assay, 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 (%)
Serum (Sample Diluent PT 1-eg)	1:4	102	92-118
	1:8	100	-
	1:16	108	101-116
	1:32	115	109-120
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

## 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).