colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human IL9 in serum, plasma and cell culture supernatants.

general information

Catalogue Number	KE00013
Product Name	IL9 ELISA Kit
Species cross-reactivity	Human IL9
Range (calibration Range)	3.13 - 200 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**3578** (Human)
SwissProt	**P15248** (Human)

kit components & storage

|		
Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard - 200 pg/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Streptavidin-HRP antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 3-ec - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

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

Sample Diluent PT 3-ec is for Standard and Samples.

Detection Diluent is for Detection antibody and streptavidin-HRP.

*Add **1 mL** Sample Diluent PT 3-ec in Standard , This reconstitution gives a stock solution of 200 pg/mL.
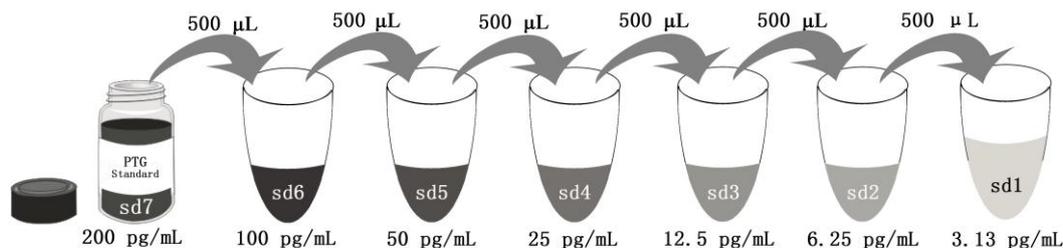
Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 3-ec	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00013 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL9 ELISA kit is to be used to detect and quantify protein levels of endogenous IL9. The assay recognizes human IL9. A monoclonal antibody specific for IL9 has been pre-coated onto the microwells. The IL9 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody of biotinylated specific for IL9 is added to detect the captured IL9 protein. For signal development, Sterptravidin-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 450nm.

background

Interleukin 9 (IL-9) is a γc-family cytokine that is highly produced by T-helper 9 (Th9) cells. IL-9 could promote the survival and activation of various cellular targets, including mast cells, B cells, T cells, and structural cells. Its expression is considered a hallmark of Th2-lineage cells. Primarily studied in Th2-type immunity, IL-9 was shown to be involved in asthma, allergy, and host defense against helminth infections. IL-9 also stimulates cell proliferation and prevents apoptosis. It functions through the interleukin 9 receptor (IL9R), which activates different signal transducer and activator (STAT) proteins and thus connects this cytokine to various biological processes.

sample preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

safety notes

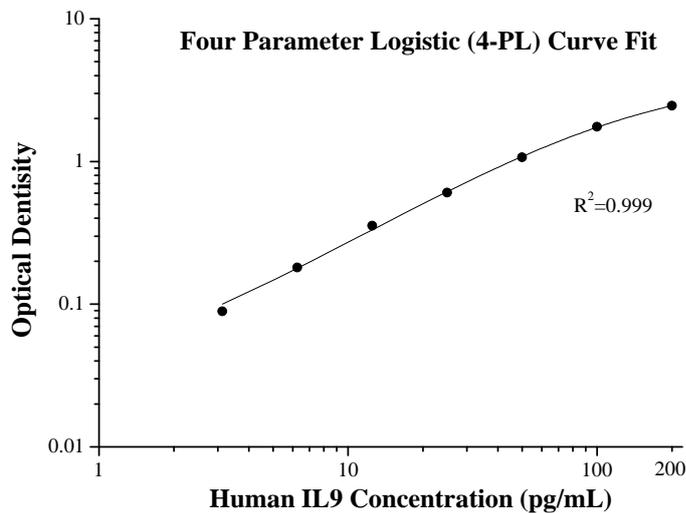This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 μL	**120 min**	4 times	Cover Wells
2	Diluent Antibody Solution	100 μL	60 min	4 times	Cover Wells
3	Diluent HRP Solution	100 μL	40 min	4 times	Cover Wells
4	TMB Substrate	100 μL	15-30 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 μL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

typical data

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

Four Parameter Logistic (4-PL) Curve Fit

$R^2=0.999$

Optical Dentisity vs Human IL9 Concentration (pg/mL)

(pg/mL)	O.D	Average	Corrected
0	0.035	0.031	—
	0.026		
3.13	0.122	0.120	0.089
	0.117		
6.25	0.211	0.211	0.181
	0.211		
12.5	0.377	0.386	0.355
	0.394		
25	0.647	0.635	0.605
	0.623		
50	1.084	1.101	1.070
	1.117		
100	1.778	1.780	1.750
	1.782		
200	2.497	2.489	2.456
	2.476		

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			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	197.3	49.2	4.1	220.7	53.6	5.9
SD	14.4	1.6	0.3	12.5	2.3	0.6
CV%	7.3	3.1	6.3	5.7	4.4	9.2

recovery

The recovery of IL9 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(%)
Citrate plasma	1:2	94	86-108
	1:4	104	87-112
Cell culture supernatants	1:2	101	79-115
	1:4	104	93-120

sample value

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human IL9 in this assay. All samples measured less than the lowest standard, 3.13 pg/mL. No medical histories were available for the donors used in this study.

sensitivity

The minimum detectable dose of human IL9 is 0.3 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, three samples were spiked with high concentrations of IL9 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

		Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	98	101
	Range (%)	92-98	96-109
1:4	Average% of Expected	99	104
	Range (%)	94-104	89-114
1:8	Average% of Expected	102	103
	Range (%)	101-103	84-119
1:16	Average% of Expected	108	104
	Range (%)	99-117	83-120

references

1. Goswami R. et al. (2011). J Immunol. 186: 3283-8.

2. Hauber HP. et al. (2004). Int Arch Allergy Immunol. 134: 79-87.

3. Temann UA. et al. (1998). J Exp Med. 188: 1307-20.

4. Soussi-Gounni. Et al. (2001). J. Allergy Clin. Immunol. 107: 575－82.

5. Provided by RefSeq, Jul 2008.

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