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colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human IL5 in serum, plasma, cell culture supernatants and urine.

general information

Catalogue Number	KE00010
Product Name	IL5 ELISA Kit
Species cross-reactivity	Human IL5
Range (calibration Range)	31.25 - 2000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**3567** (Human)
SwissProt	**P05113** (Human)

kit components & storage

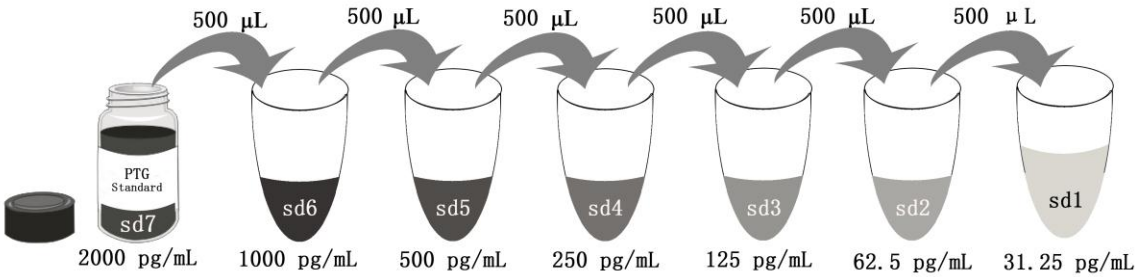
Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard - 4000 pg/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-a - 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 1-a is for Standard and Samples.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

*Add 2 mL Sample Diluent PT 1-a 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-a	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE000010 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL5 ELISA kit is to be used to detect and quantify protein levels of endogenous IL5. The assay recognizes human IL5. A polyclonal antibody specific for IL5 has been pre-coated onto the microwells. The IL5 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for IL5 is added to detect the captured IL5 protein. For signal development, horseradish peroxidase (HRP)-conjugated Anti-mouse antibody 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-5, or IL5, was originally discovered as a soluble T cell-derived factor, called T cell-replacing factor (TRF), that induced T cell-depleted activated B cells to secrete immunoglobulin. IL5 is a key hematopoietic cytokine in eosinophil differentiation, maturation, recruitment and activation at sites of allergic inflammation. IL5 also plays a role in the development, metabolism, and function of basophils. IL5 exerts its biological activity through the IL5 receptor (IL5R), which is composed of at least two chains: an α chain that binds IL5 with low affinity and a β chain that does not bind IL5, but together with the IL5α chain, constitutes the high affinity IL5 receptor. The β chain is common to the IL-3, IL5 and GM-CSF receptors and has been shown to signal through the JAK/Stat pathway. IL5 has long been associated with the cause of several allergic diseases including allergic rhinitis and asthma, wherein a large increase in the number of circulating, airway tissue, and induced sputum eosinophils have been observed.

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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

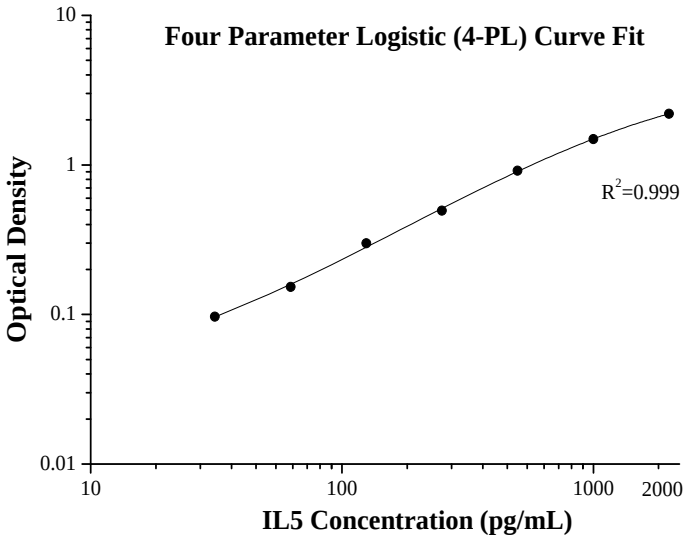
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.



(pg/mL)	O.D	Average	Corrected
0	0.087	0.087	—
	0.087		
31.25	0.169	0.1835	0.0965
	0.198		
62.5	0.25	0.2395	0.1525
	0.229		
125	0.41	0.3855	0.2985
	0.361		
250	0.606	0.5805	0.4935
	0.555		
500	1.003	1.0025	0.9155
	1.002		
1000	1.585	1.574	1.487
	1.563		
2000	2.239	2.2855	2.1985
	2.332		

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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)	903.6	426	39.3	977	475.9	44.5
SD	59.1	36.3	3.8	65.9	32.1	4.3
CV%	6.5	8.5	9.7	6.7	6.7	9.7

recovery

The recovery of IL5 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	102	87-116
	1:4	92	87-103
Cell culture supernatants	1:2	104	92-113
	1:4	100	91-116
Urine	1:2	99	87-123
	1:4	96	82-112

sample values

Thirty-two serum and plasma samples from healthy volunteers were evaluated for human IL5 in this assay. All samples measured less than the lowest standard, 31.25 pg/mL. No medical histories were available for the donors used in this study.

sensitivity

The minimum detectable dose of human IL5 is 30 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 IL5 in various matrices and diluted with the appropriate **Sample Diluent PT 1-a** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:1)

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		Citrate plasma	Cell culture supernatants	Urine
1:2	Average% of Expected	81	94	79
	Range(%)	80-83	88-106	78-79
1:4	Average% of Expected	94	94	88
	Range(%)	85-99	83-116	86-89
1:8	Average% of Expected	98	93	102
	Range(%)	85-114	80-113	101-102
1:16	Average% of Expected	98	96	115
	Range(%)	87-117	86-109	107-123

references

1. Sehmi R et al. (1992) Blood.79: 2952-9.
2. Yamaguchi Y et al. (1988) J Exp Med. 167: 1737-42.
3. Kouro T et al. (2009) Int Immunol. 21: 1303-9.
4. Tavernier J et al. (1991) Cell. 66:1175-84.
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