

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of mouse Interleukin 23 (IL23) concentrations in serum, plasma, and cell culture supernatants.

general information

Catalogue Number	KE10013
Product Name	IL23 ELISA Kit
Species cross-reactivity	Mouse IL23
Range (calibration Range)	15.6 - 1000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	83230 / 16160 (Mouse)
SwissProt	Q9EQ14 / P43432 (Mouse)

kit components & storage

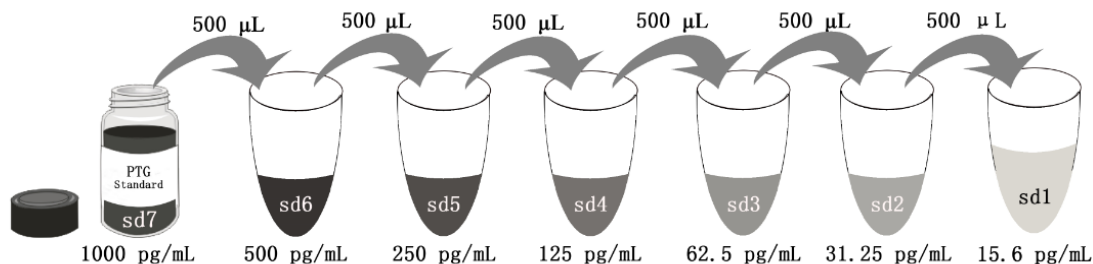
Microplate - antibody coated 96-well Microplate (8 wells x12 strips)	1 plate	Store at -20°C for six months
Standard - 1000 pg/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) , biotinylated - 150 µL/vial	1 vial	Store at 2-8°C for six months
Streptavidin-HRP (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-ef - 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-ef is for Standard and Samples.

Detection Diluent is for detection antibody and streptavidin-HRP.

*Add 1 mL Sample Diluent PT 1-ef in Standard, This reconstitution gives a stock solution of 1000 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-ef	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

K10013 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The mouse IL23 ELISA kit is to be used to detect and quantify protein levels of endogenous mouse IL23. The assay recognizes mouse IL23 complex which has two subunits IL23p19 and IL12p40. A monoclonal antibody specific for mouse IL23p19 has been pre-coated onto the microwells. The mouse IL23 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody of biotinylated specific for mouse IL12p40 is added to detect the captured mouse IL23 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 450nm.

background

Interleukin 23 (il-23) is a member of the il12 cytokine family and composed of two subunits, il12p40 and il23p19. It is produced by antigen presenting cells and has been shown to promote the production and survival of a distinct lineage of T-cells called TH17 cells. A functional receptor for il-23 (the il-23 receptor) has been identified and is composed of Il-12R β 1 and Il-23R. Il-23 is expressed chiefly by the macrophages and DCs. The il-23R is found on memory T cells, NKT cells, macrophages, DCs, and naive T cells upon activation by TGF-β and IL-6. The main biological effects of il-23 identified initially consist of stimulation of antigen presentation by DCs, T cell differentiation to Th17 cells, and production of interferon-γ (IFN-γ). Il-23 acts also as an end-stage effector cytokine through direct action on macrophages.

sample preparation

The serum, plasma or cell culture supernatants 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

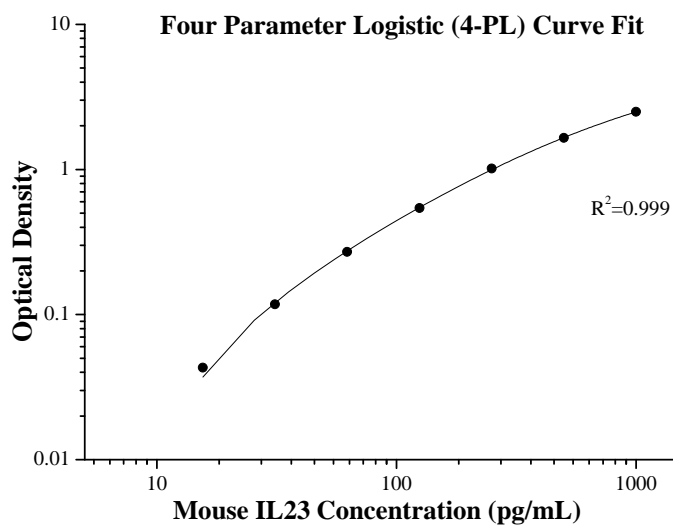
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.098	0.098	0
	0.098		
15.6	0.139	0.141	0.043
	0.143		
31.25	0.214	0.216	0.118
	0.217		
62.5	0.370	0.368	0.270
	0.366		
125	0.627	0.639	0.541
	0.651		
250	1.108	1.112	1.014
	1.115		
500	1.749	1.747	1.649
	1.745		
1000	2.573	2.595	2.497
	2.616		

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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	818.2	222.5	20.3	829.1	187.0	21.1
SD	35.69	9.77	1.78	56.29	8.66	0.89
CV%	4.4	4.4	8.8	6.8	4.6	4.2

recovery

The recovery of IL23 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(%)
Mouse serum	1:2	83	80-86
	1:4	94	88-100
Cell culture supernatants	1:2	104	84-106
	1:4	114	104-123

sensitivity

The minimum detectable dose of mouse IL23 is 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 mouse IL23 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Serum	Cell culture supernatants
1:2	Average% of Expected	85	118
	Range(%)	80-92	105-119
1:4	Average% of Expected	117	113
	Range(%)	111-124	102-115
1:8	Average% of Expected	-	98
	Range(%)	-	94-105
1:16	Average% of Expected	-	95
	Range(%)	-	82-101

references

1. Kleinschek MA. et al.(2006). J Immunol. 176:1098-106.
2. Langrish CL. et al.(2005). J Exp Med. 201:233-40.
3. Parham C. et al.(2002). Journal of Immunology. 168 (11): 5699-708.
4. Cua DJ. et al. (2003). Nature.421: 744-8.