

Mouse IL-1ra/IL-1F3 Sandwich ELISA Kit Datasheet

For the quantitative detection of mouse IL-1ra concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE10052
Product Name	Mouse IL-1ra/IL-1F3 Sandwich ELISA Kit
Species cross-reactivity	Mouse
Range (calibration Range)	15.6-1000 pg/mL, 7.8-500 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	16181
SwissProt	P25085

Kit Components & Storage

Microplate - antibody coated 96 - well microplate (8 well × 12 strips)	1 plate	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 - 1000 pg/bottle; lyophilized*Reconstitution by Sample Diluent PT 3-eg	2 bottles	
Protein standard - 1500 pg/bottle; lyophilized*Reconstitution by Sample Diluent PT 5-ef	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 3-eg - 30 mL/bottle. For serum and plasma	1 bottle	
Sample Diluent PT 5-ef - 30 mL/bottle. For cell culture supernatants	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.

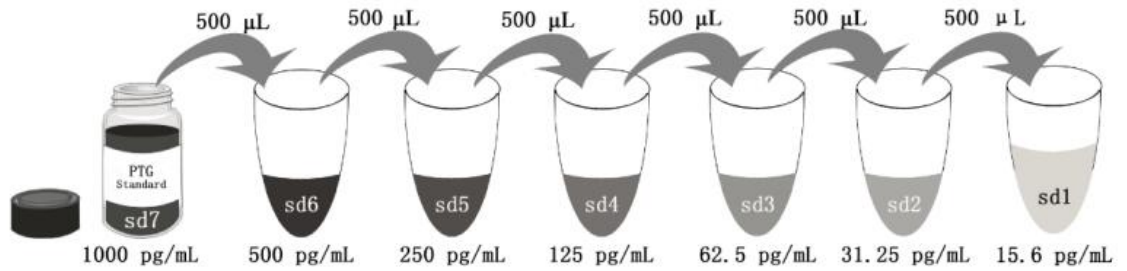
Sample Diluent PT 3-eg is for protein standard, serum and plasma.

Sample Diluent PT 5-ef is for protein standard and cell culture supernatants.

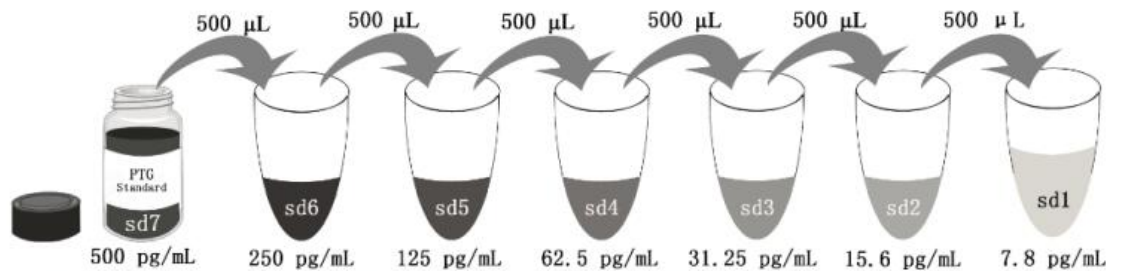
Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 1 mL Sample Diluent PT 3-eg in protein standard. This reconstitution gives a stock solution of 1000 pg/mL.

*Add 3 mL Sample Diluent PT 5-ef in protein standard. This reconstitution gives a stock solution of 500 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-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 5-ef	3000 μL	500 μL	500 μL	500 μL	500 μL	500 μL	500 μL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE10052 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The IL-1ra ELISA kit is to be used to detect and quantify protein levels of endogenous IL-1ra. The assay recognizes human, mouse and Rat IL-1ra. An antibody specific for IL-1ra has been pre-coated onto the microwells. The IL-1ra protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for IL-1ra is added to detect the captured IL-1ra 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

IL-1Ra, also known as IL-1F3, is a member of the interleukin 1 cytokine family. It is a receptor antagonist of IL-1, can competitively bind with IL-1R1 thereby blocking cell activation by the cytokine. IL-1Ra is secreted by various types of cells including immune cells, epithelial cells, and adipocytes, and is a natural inhibitor of the pro-inflammatory effect of IL-1 β . This protein inhibits the activities of interleukin 1, alpha (IL1A) and interleukin 1, beta (IL1B), and modulates a variety of interleukin 1 related immune and inflammatory responses, particularly in the acute phase of infection and inflammation. As a potent immune/inflammatory molecule, IL-1Ra is tightly associated with osteomyelitis, rheumatoid arthritis, traumatic brain injury (TBI) and stroke. Recently, an increasing number of studies proved that IL-1Ra is also actively involved in tumor progression, such as high expression IL-1Ra increased the risk of bladder cancer and non-cardia gastric carcinoma.

Sample Preparation

The samples may require proper dilution to fall within the range of the assay. 1:2 or 1:4 dilution is recommended for serum or plasma. 1:4 or 1:8 dilution is recommended for cell culture supernatants.

Safety Notes

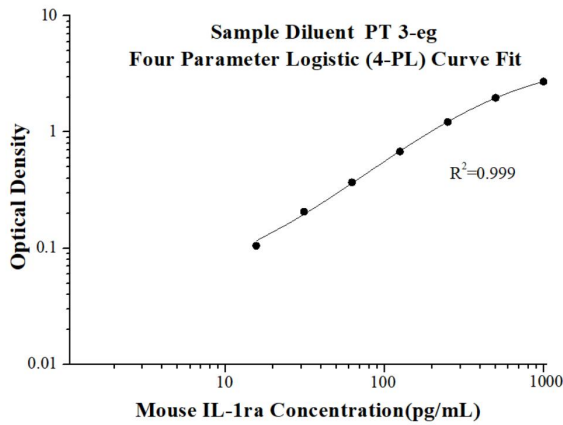
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

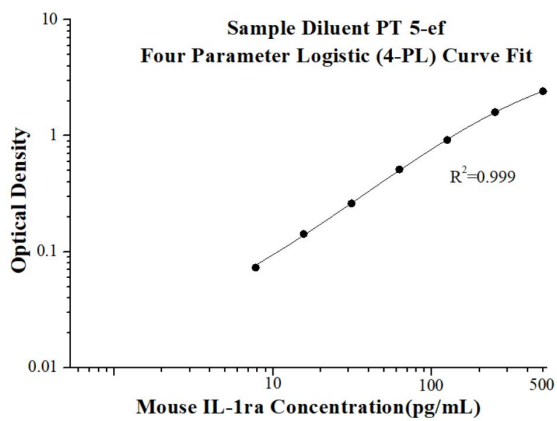
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 μ L	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 μ L	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 μ L	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 μ L	15-20 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.				

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	0.027 0.027	0.027	-
15.6	0.137 0.127	0.132	0.105
31.25	0.238 0.229	0.234	0.207
62.5	0.397 0.395	0.396	0.369
125	0.720 0.695	0.708	0.681
250	1.299 1.197	1.248	1.221
500	1.999 1.996	1.998	1.971
1000	2.757 2.724	2.741	2.714



(pg/mL)	O.D	Average	Corrected
0	0.048 0.045	0.047	-
7.8	0.117 0.122	0.120	0.073
15.6	0.187 0.190	0.189	0.142
31.25	0.302 0.312	0.307	0.261
62.5	0.549 0.568	0.559	0.512
125	0.955 0.976	0.966	0.919
250	1.638 1.649	1.644	1.597
500	2.448 2.482	2.465	2.419

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	100.4	2.4	2.4
2	20	190.5	4.8	2.5
3	20	605.6	18.4	3.0

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	103.0	3.0	3.0
2	24	195.9	7.8	4.0
3	24	598.0	15.5	2.6

Recovery

The recovery of IL-1ra 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	1:8	97	78-110
	1:16	100	79-120
cell culture supernatants	1:4	100	81-118
	1:8	97	79-106

Sample Values

Sample Type	Mean (pg/mL)	Range (pg/mL)
Mouse plasma (n=23)	870	307-2001

Sensitivity

The minimum detectable dose of IL-1ra is 3.0 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, cell culture supernatants were spiked with high concentrations of IL-1ra in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

Mouse serum 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 (%)
Mouse serum (PT 3-eg)	1:2	100	-
	1:4	96	95-97
	1:8	103	94-107
	1:16	106	93-112
cell culture supernatants (PT 5-ef)	1:4	105	95-123
	1:8	108	96-125
	1:16	108	101-116
	1:32	110	101-115

References

1. Stephane Perrier et al. (2006) FEBS Lett. 580(27):6289-94.
2. Charles A. Dinarello. et al. (2020) Immunol Rev. 281(1):8-27.
3. Ivona Aksentijevich. et al.(2009) N Engl J Med. 360(23):2426-37.
4. C Grond-Ginsbach. et al.(2008) J Neurol. 255(5):723-31.
5. Gifone Aguiar Rocha. et al. (2005) Int J Cancer. 115(5):678-83.
6. Zhibin Hu. et al. (2006) Cancer Lett. 236(2):269-75.