

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of mouse MIF in serum, plasma and cell culture supernatants.

general information

Catalogue Number	KE10027
Product Name	MIF ELISA Kit
Species cross-reactivity	Mouse MIF
Range (calibration Range)	31.25 – 2000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	17319 (Mouse)
SwissProt	P34884 (Mouse)

kit components & storage

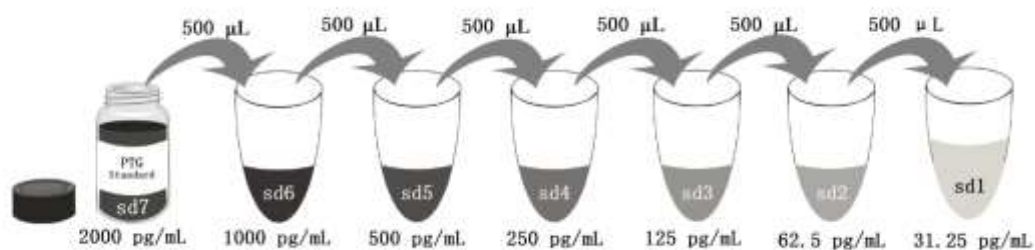
Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard – 2000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection Antibody (100X) , biotinylated - 120 µL/vial	1 vial	Store at 2-8°C for six months
Streptavidin-HRP (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1- 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 is for standard and samples.

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

*Add 1 mL Sample Diluent PT 1 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	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE10027 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The MIF ELISA kit is to be used to detect and quantify protein levels of endogenous MIF. The assay recognizes mouse MIF. An antibody specific for MIF has been pre-coated onto the microwells. The MIF protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for mouse MIF is added to detect the captured mouse MIF 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 with the correction wavelength set at 630 nm.

background

Macrophage migration inhibitory factor (MIF) is an upstream regulator of the innate and adaptive immune response that is widely expressed and that exhibits chemokine-like activities. MIF play a role in the pathogenesis of inflammatory diseases, such as atherosclerosis, rheumatoid arthritis, sepsis, asthma, and acute respiratory distress syndrome. MIF is expressed by the ischemic myocardium and that it may exhibit an overall cardioprotective role during I/R injury. MIF regulates inflammatory processes by recruiting immune cells, amplifying cytokine production, inhibiting apoptosis and counter-regulating the immunosuppressive action of glucocorticoids.

sample preparation

The serum ,plasma and cell culture supernatants may require proper dilution to fall within the range of the assay. The serum samples are suggested diluted 1:400. The cell culture supernatants are suggested diluted 1:25

safety notes

This product is sold for lab research and development use ONLY and not for use in humans or animals.

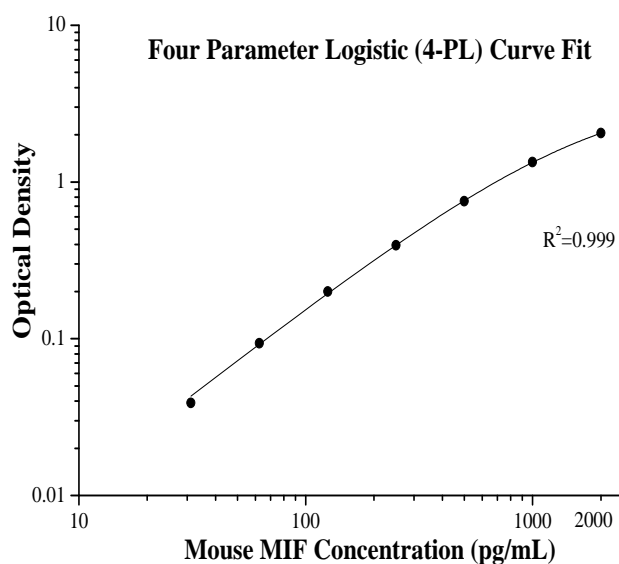
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 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	0.088	0.091	—
	0.094		
31.25	0.131	0.13	0.039
	0.29		
62.5	0.188	0.185	0.094
	0.181		
125	0.298	0.291	0.2
	0.284		
250	0.5	0.486	0.395
	0.471		
500	0.857	0.846	0.755
	0.853		
1000	1.438	1.428	1.337
	1.418		
2000	2.145	2.140	2.05
	2.134		

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)	47.1	423.9	1,663.7	40.6	391.2	1,645.0
SD	2.9	16.5	99.9	2.7	25.0	107.9
CV%	6.2	3.8	6.0	6.5	6.3	6.5

recovery

The recovery of MIF 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	1:800	103	91-117
	1:1,600	105	87-112
Cell culture supernatants	1:50	108	87-119
	1:100	109	86-119

sample value

Eight individual mouse serum samples were evaluated for the presence of mouse MIF in this assay.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Mouse serum (n=8)	235	166-292

sensitivity

The minimum detectable dose of mouse MIF is 2.3 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, samples containing concentration of mouse MIF were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

(The serum samples were initially diluted 1:400. The cell culture supernatants were initially diluted 1:25)

		Mouse serum	Cell culture supernatants
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	107	115
	Range (%)	106-108	114-116
1:8	Average% of Expected	119	123
	Range (%)	116-123	123-124
1:16	Average% of Expected	120	127
	Range (%)	117-123	126-128

references

1. Calandra T. et al. (2003). *Nat Rev Immunol.* 3(10):791-800.
2. Koga K. et al.(2011). *Antioxid Redox Signal.* 14(7):1191-202.
3. Qi D. et al.(2009). *J Clin Invest.* 119(12):3807-16.
4. Stoppe C. et al.(2012). *Mol Med.*18:843-50.
5. Rajasekaran D. et al.(2014). 28(11):4961-71.
6. Bernhagen J. et al. (2007). 13(5):587-96.