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colorimetric sandwich ELISA kit datasheet

For the quantitative detection of mouse/rat IGF1 concentrations in serum, plasma, cell lysates and tissue lysates.

general information

Catalogue Number	KE10032
Product Name	IGF1 ELISA Kit
Species cross-reactivity	Mouse / Rat IGF1
Range (calibration Range)	15.6 - 1000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**16000 (Mouse)/24482 (Rat)**
SwissProt	**P05017 (Mouse)/P08025 (Rat)**

kit components & storage

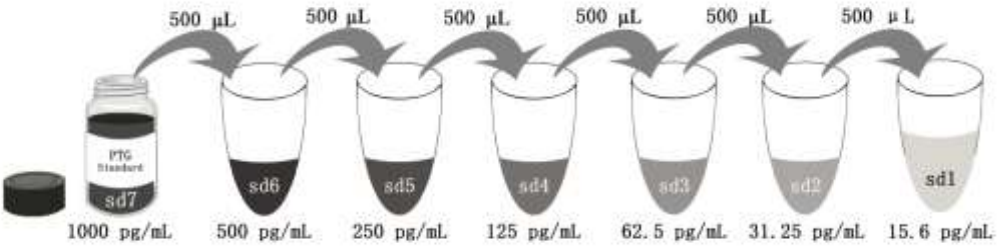
Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard - 1000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Streptavidin-HRP (100X) - 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
Extraction Reagent - 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.

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

product description

KE10032 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The Mouse/Rat IGF1 ELISA kit is to be used to detect and quantify protein levels of endogenous mouse/rat IGF1. The assay recognizes mouse/rat IGF1. An antibody specific for mouse/rat IGF1 has been pre-coated onto the microwells. The mouse/rat IGF1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for mouse/rat IGF1 is added to detect the captured mouse/rat IGF1 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

Insulin-like-growth factor 1 (IGF1), a 70 amino-acid peptide hormone is the principal mediator of biochemical effects of growth hormone (GH). IGF1 is an important growth factor in the regulation of cell proliferation and differentiation. IGF1 is largely synthesized in the liver (75%) and, to a lesser extent, in peripheral tissues. IGF1 have been shown to play an essential role in preventing the formation of fatty liver. IGF1 is a potent mitogen and is inhibited by IGF-binding protein-3 (IGFBP3). High serum IGF1 and low IGFBP3 are associated with increased risk of several carcinomas. Mice lacking IGF1 exhibit generalized organ hypoplasia including underdevelopment of the central nervous system and developmental defects in bone, muscle and reproductive systems.

sample preparation

Different samples may require proper dilution to fall within the range of the assay. The serum or plasma is better to be diluted 1:1000 or 1:2000 before assay, 1:4 or 1:8 dilution is recommended for cell lysates samples and tissue lysates samples.

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safety notes

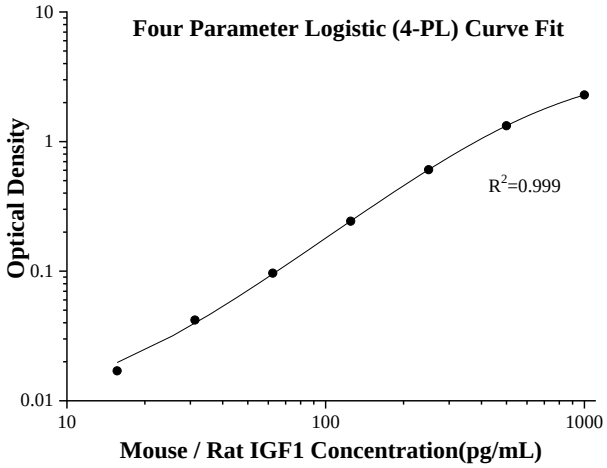
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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	0.042	0.042	—
	0.041		
15.6	0.059	0.059	0.017
	0.058		
31.25	0.084	0.084	0.042
	0.083		
62.5	0.138	0.138	0.097
	0.138		
125	0.284	0.285	0.243
	0.285		
250	0.653	0.648	0.607
	0.643		
500	1.384	1.367	1.325
	1.349		
1000	2.352	2.333	2.292
	2.314		

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.

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	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	550.2	164.8	78.0	501.3	138.5	56.0
SD	16.0	6.7	5.1	38.4	14.1	6.1
CV%	2.9	4.1	6.5	7.7	10.2	10.8

recovery

The recovery of IGF1 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	1:1000	104	95-113
	1:2000	106	98-119
Rat serum	1:1000	109	90-117
	1:2000	97	79-114
Tissue lysates	1:16	104	87-121
	1:32	101	99-103

sample values

Serum -Six indicidual rat serum samples and ten individual mouse serum samples were evaluated for the presence of mouse/rat IGF1 in this assay.

Sample Type	Mean of Detectable (**ng/mL**)	Range (**ng/mL**)
Mouse serum (n=10)	437.6	217.4-656.4
Rat serum (n=6)	510.9	274.2-821.3

Tissue lysates -Dissect the tissue of interest and wash briefly with chilled **1X PBS** to remove any blood if necessary, cut the tissue into smaller pieces whilst keeping it on ice. Transfer the tissue to a homogenizer and add **Extraction Reagent** with protease inhibitor. In general, add 500 µL **Extraction Reagent** for approximately every 10 mg of tissue. Homogenize thoroughly and keep the sample on ice for 30 min. Sonicate the sample and centrifuge at 10,000 x g, then transfer the supernatant to assay.

	IGF1 (pg/mL)	Total protein (mg/mL)
Mouse lung	4,677.6	5.6
Rat heart	3,862.3	10.8

*__1X PBS__ For 1000 mL

10 mM Na_2HPO4, 1.8 mM NaH_2PO4, 140 mM NaCl. Adjust pH to 7.4 and add ddH_2O to 1000 mL.

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sensitivity

The minimum detectable dose of mouse/rat IGF1 is 1.3 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 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 1:500. The cell lysates samples and tissue lysates samples were initially diluted 1:2.)

		Mouse serum	Rat serum	Tissue lysates
1:2	Average% of Expected	100	100	100
	Range (%)	99-101	99-101	99-101
1:4	Average% of Expected	100	103	106
	Range (%)	98-101	96-110	105-107
1:8	Average% of Expected	101	107	108
	Range (%)	101-102	96-113	103-115
1:16	Average% of Expected	100	110	118
	Range (%)	95-104	103-124	104-128

references

1. Adamo ML. et al. (1993) Adv Exp Med Biol. 343:1-11.
2. Adachi Y. et al. (2019) J Gastroenterol Hepatol. 2019 Jun 3.
3. Anisimov VN. et al. (2019) Crit Rev Oncol Hematol. 87(3):201-23.
4. Sonntag WE. et al. (2012) J Gerontol A Biol Sci Med Sci. 67(6):587-98.

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