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Antibodies | ELISA kits | Proteins

AuthentiKine™

Human HGF sandwich ELISA kit datasheet

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

general information

Catalogue Number	KE00168
Product Name	AuthentiKine Human HGF ELISA Kit
Species cross-reactivity	Human HGF
Range (calibration Range)	0.313 - 20 ng/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**3082** (Human)
SwissProt	**P14210** (Human)

kit components & storage

Microplate - antibody coated 96 - well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Protein standard - 40 ng/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody, HRP-conjugated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-ac - 30 mL/bottle. For serum, plasma samples	1 bottle	Store at 2-8°C for six months
Sample Diluent PT 1-ec - 30 mL/bottle. For cell culture supernatants	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	

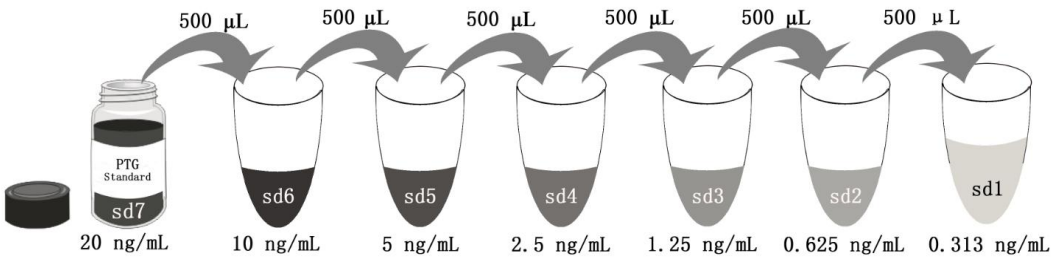
NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-ac is for protein standard, serum and plasma samples.

Sample Diluent PT 1-ec is for protein standard and cell culture supernatants.

Detection Diluent is for Detection antibody.

*Add **2 mL** Sample Diluent PT 1-ac or PT 1-ec in protein standard. This reconstitution gives a stock solution of 20 ng/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-ac or PT 1-ec	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00168 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The HGF ELISA kit is to be used to detect and quantify protein levels of endogenous HGF. The assay recognizes human HGF. An antibody specific for HGF has been pre-coated onto the microwells. The HGF protein in samples is captured by the coated antibody after incubation. Following extensive washing, the horseradish peroxidase (HRP)-conjugated detection antibody specific for HGF is added to detect the captured HGF protein. For signal development, 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

Hepatocyte growth factor (HGF) is the most potent mitogen of mature hepatocytes in primary culture. HGF is derived from a biologically inactive single chain precursor of 728 amino acids (pro-HGF) localized mostly on the cell surface and in the extracellular matrix. The mature form produced following proteolytic cleavage is composed of a 69-kDa alpha-subunit (containing four kringle domains) and the 34 kDa beta-subunit, similar to the catalytic domain of serine proteases, but with amino acid substitutions in the active site. HGF is a pleiotropic cytokine which exerts a variety of effects on several cells, being involved in the regulation of many biological processes, such as inflammation, tissue repair, morphogenesis, angiogenesis, tumour propagation, immunomodulation of viral infections and cardio-metabolic activities.

sample preparation

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

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.

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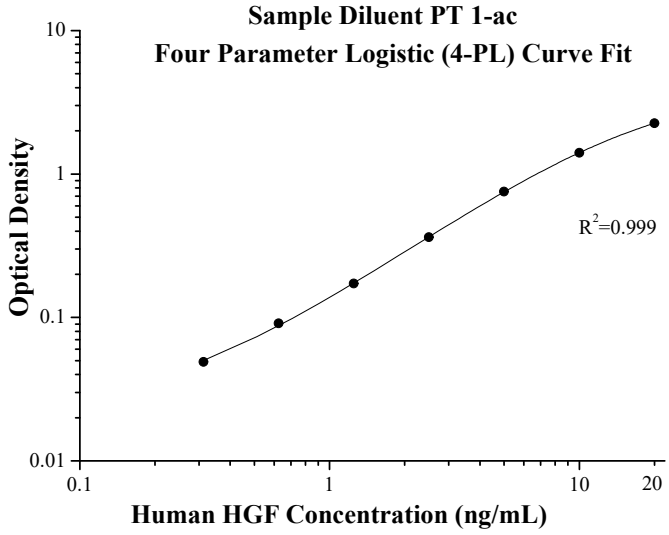
assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	**120 min**	4 times	Cover Wells incubate at 37°C
2	Diluent Detection antibody, HRP-conjugated Solution	100 µL	**40 min**	4 times	Cover Wells incubate at 37°C
3	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37℃
4	Stop Solution	100 µL	0 min	Do not wash	-
5	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.



(ng/mL)	O.D	Average	Corrected
0	0.084	0.082	—
	0.08		
0.313	0.126	0.131	0.049
	0.136		
0.625	0.172	0.173	0.091
	0.173		
1.25	0.256	0.254	0.172
	0.252		
2.5	0.431	0.444	0.362
	0.456		
5	0.832	0.835	0.753
	0.838		
10	1.458	1.488	1.406
	1.517		
20	2.349	2.343	2.261
	2.336		

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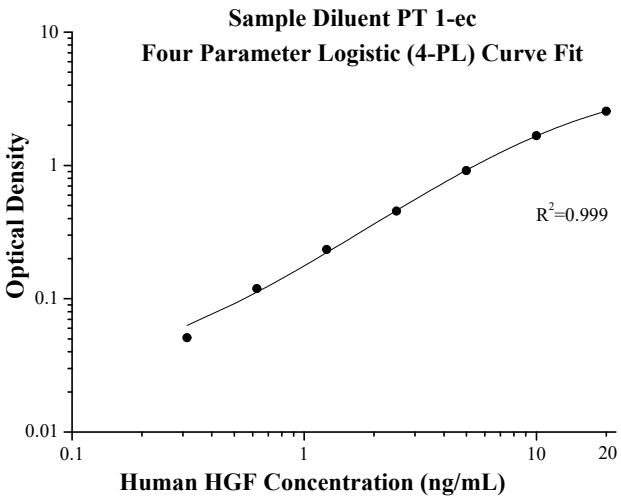

(ng/mL)	O.D	Average	Corrected
0	0.076	0.075	—
	0.074		
0.313	0.129	0.126	0.051
	0.123		
0.625	0.19	0.194	0.119
	0.197		
1.25	0.313	0.309	0.234
	0.304		
2.5	0.53	0.530	0.455
	0.53		
5	0.958	0.989	0.914
	1.019		
10	1.797	1.744	1.669
	1.69		
20	2.611	2.628	2.553
	2.644		

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 (ng/mL)	4.7	9.3	18.3	5.1	10.5	20.9
SD	0.30	0.65	0.92	0.42	0.96	1.93
CV%	6.4	7.0	5.0	8.4	9.2	9.2

recovery

The recovery of HGF spiked to three different levels in four samples throughout the range of the assay in human samples were evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:4	87	73-111
	1:8	95	80-112
Human plasma	1:4	98	90-112
	1:8	95	79-111
Cell culture supernatants	1:2	119	114-126
	1:4	114	93-125

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sample values

Sixteen Serum Samples from healthy volunteers were evaluated for HGF in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Human serum (n=16)	2.4	94	0.9-4.7

THP-1 human acute monocytic leukemia cells were cultured to a density of 1 x10^6/mL in RPMI 1640 supplemented with 10% fetal bovine serum and 50 µM β-mercaptoethanol. Aliquots of the cell culture supernates were removed, assayed for human HGF, and measured 0.4 ng/ml.

sensitivity

The minimum detectable dose of human HGF is 0.26 ng/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, serum samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. Cell culture supernatants samples were spiked with high concentrations of Human HGF and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

		Human serum (Sample Diluent PT 1-ac)	Cell culture supernatants (Sample Diluent PT 1-ec)
1:2	Average% of Expected	100	102
	Range (%)	-	102-103
1:4	Average% of Expected	96	107
	Range (%)	87-108	104-110
1:8	Average% of Expected	96	96
	Range (%)	78-115	90-103
1:16	Average% of Expected	97	94
	Range (%)	72-122	93-95

references

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Bardelli A. et al. (1994). J Biotechnol. 37(2):109-22.
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