

Human Growth Hormone Sandwich ELISA Kit Datasheet

For the quantitative detection of Human Growth Hormone in serum, plasma and cell culture supernatants .

General Information

Catalogue Number	KE00221
Product Name	AuthentiKine™ Human Growth Hormone Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	3.9-250 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	2688
SwissProt	P01241

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 - 500 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 3 - 30 mL/bottle. For human serum and plasma	1 bottle	Store at 2-8°C for six months
Sample Diluent PT 1-ef - 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	

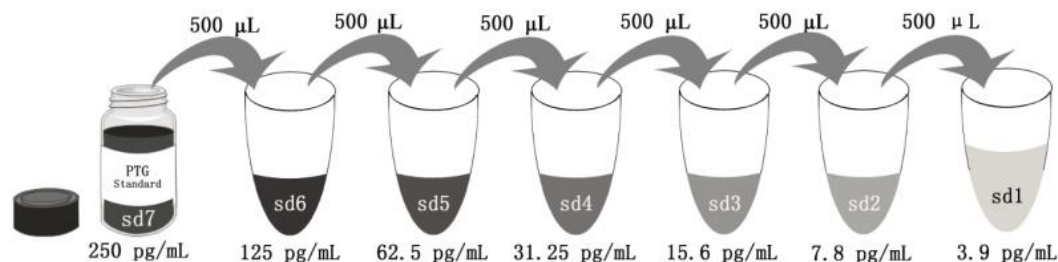
NB: Do not use the kit after the expiration date.

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

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

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

*Add 2 mL Sample Diluent PT 1-ef or PT 3 in protein standard. This reconstitution gives a stock solution of 250 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 or PT1-ef	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00221 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The ELISA kit is to be used to detect and quantify protein levels of endogenous HGH. The assay recognizes human HGH An antibody specific for HGH has been pre-coated onto the microwells. The HGH protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for HGH is added to detect the captured HGH protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody 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

Growth hormone (GH) is a heterogeneous polypeptide consisting of a number of different isoforms and variants. Its secretion from pituitary somatotrophs is pulsatile, with low or undetectable basal levels between peaks. GH secretion has a characteristic diurnal pattern with maximal levels recorded during sleep. Growth hormone is a powerful anabolic hormone that stimulates growth, cell reproduction, and cell regeneration in humans and other animals. The actions of GH are mediated by GH receptors (GHR), which are widely distributed among tissues. Mutations in or deletions of the gene lead to growth hormone deficiency and short stature.

Sample Preparation

Samples may require proper dilution to fall within the range of the assay. 1:2 to 1:16 dilution is recommended for serum and plasma.

Safety Notes

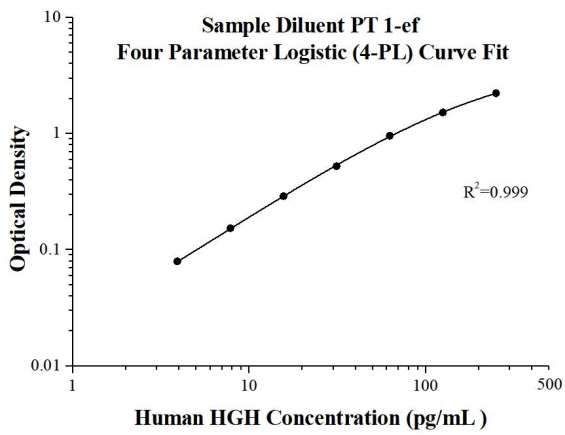
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

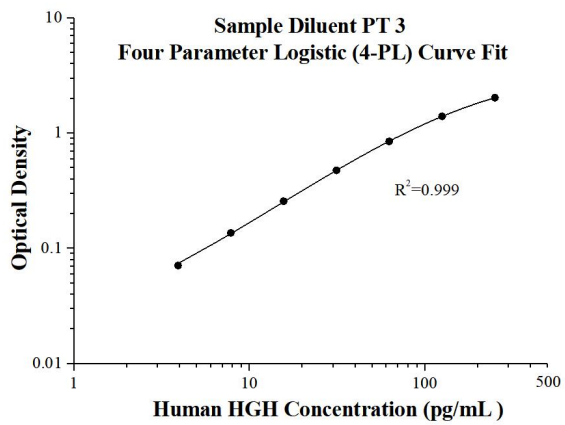
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min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

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	0.065 0.074	0.070	0
3.9	0.141 0.157	0.149	0.080
7.8	0.211 0.235	0.223	0.154
15.6	0.346 0.373	0.360	0.290
31.2	0.570 0.618	0.594	0.525
62.5	1.000 1.058	1.029	0.960
125	1.509 1.673	1.591	1.522
250	2.259 2.337	2.298	2.229



(pg/ml)	O.D	Average	Corrected
0	0.069 0.071	0.070	0
3.9	0.139 0.143	0.141	0.071
7.8	0.207 0.206	0.207	0.137
15.6	0.323 0.331	0.327	0.257
31.2	0.542 0.551	0.547	0.477
62.5	0.895 0.946	0.921	0.851
125	1.458 1.491	1.475	1.405
250	2.087 2.121	2.104	2.034

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	n	Mean (pg/ml)	SD	CV%	Sample	n	Mean (pg/ml)	SD	CV%
1	20	109.2	2.1	1.9	1	24	103.4	7.6	7.3
2	20	24.2	0.7	2.7	2	24	23.2	1.8	7.9
3	20	5.3	0.3	6.4	3	24	13.5	0.6	4.8

Recovery

The recovery of HGH 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 (%)
Human serum	1:2	78	73-87
	1:4	87	83-91
Cell culture supernatants	1:2	80	73-86
	1:4	79	73-91

Sample Values

Samples from healthy volunteers were evaluated for Growth Hormone in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Females serum (n=16)	1469.9	100	24.8-3024.8
Males serum (n=16)	592.5	100	29.7-3330.2

Human peripheral blood mononuclear cells (1×10^6 cells/mL) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 ug/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 100 ug/mL PHA. Aliquots of the cell culture supernates were removed 3d and assayed for levels of human Growth Hormone. No detectable levels were observed.

Sensitivity

The minimum detectable dose of human HGH is 0.46 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.

		Human serum (PT 3)	Cell culture supernatants (PT 1-ef)
1:2	Average% of Expected	100	93
	Range (%)	-	86-101
1:4	Average% of Expected	108	97
	Range (%)	98-113	92-104
1:8	Average% of Expected	117	99
	Range (%)	113-119	97-100
1:16	Average% of Expected	119	103
	Range (%)	118-120	103-104

References

1. Strobl JS. et al.(1994) Pharmacol Rev. 46(1):1-34.
2. Baumann GP. et al. (2009) Growth Horm IGF Res. 19(4):333-40.
3. Catchpole HR. et al. (1980) JAMA. 243(7):652.
4. Murray RD. et al. (2000) Expert Opin Pharmacother.1(5):975-90