colorimetric sandwich ELISA kit datasheet

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

general information

Catalogue Number	KE00101
Product Name	Hemopexin ELISA Kit
Species cross-reactivity	Human Hemopexin
Range (calibration Range)	62.5 - 4000 ng/mL
Tested applications	Quantification ELISA

database links

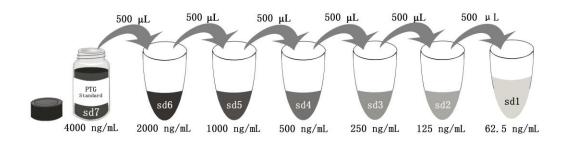| Entrez Gene	3263 (Human)
SwissProt	P02790 (Human)

kit components & storage

Microplate - antibody coated 96-well Microplate (8 wells × 12 strips)	1 plate	Store at 2-8°C for six months
Standard - 4000 ng/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 1-ef - 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-ef is for Standard and samples.

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

*Add 1 mL Sample Diluent PT 1-ef in Standard, This reconstitution gives a stock solution of 4000 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-ef	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00101 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The Hemopexin ELISA kit is to be used to detect and quantify protein levels of endogenous Hemopexin. The assay recognizes human Hemopexin. A polyclonal antibody specific for Hemopexin has been pre-coated onto the microwells. The Hemopexin protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for Hemopexin is added to detect the captured Hemopexin 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 450nm.

background

Hemopexin (HPX) is the plasma protein responsible for scavenging heme, thus preventing heme-mediated oxidative stress and heme-bound iron loss. In addition, hemopexin blocks heme activation of immune receptors and vascular inflammatory processes. It is mainly expressed in liver, the synthesis of which is induced after inflammation. Alterations of plasma hemopexin level have been linked to disorders like atherosclerosis and inflammatory diseases.

sample preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:50, 1:100 is suggested according to the individual samples.
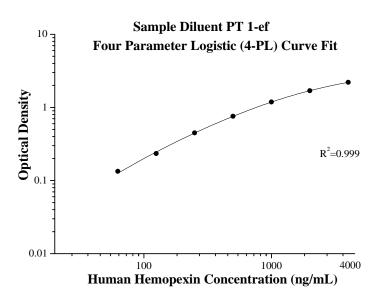
safety notes

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
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells
4	TMB Substrate	100 µL	15-30 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.

Sample Diluent PT 1-ef
Four Parameter Logistic (4-PL) Curve Fit

R^2=0.999

Optical Density vs Human Hemopexin Concentration (ng/mL)

(ng/mL)	O.D	Average	Corrected
0	0.103	0.101	—
	0.099		
62.5	0.228	0.235	0.134
	0.242		
125	0.33	0.335	0.234
	0.34		
250	0.567	0.549	0.448
	0.531		
500	0.845	0.859	0.758
	0.873		
1000	1.313	1.291	1.190
	1.268		
2000	1.809	1.79	1.689
	1.771		
4000	2.284	2.309	2.208
	2.333		

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)	209.5	842.4	3480.2	231.4	1016.8	3776.3
SD	19.82	76.08	287.76	18.17	77.66	321.93
CV%	9.5	9.0	8.3	7.9	7.6	8.5

recovery

The recovery of Hemopexin 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(%)
Citrate plasma	1:50	91	78-103
	1:100	107	97-121
Cell culture supernatants	1:2	93	77-121
	1:4	93	79-121

sample value

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human Hemopexin in this assay. All samples measured between 55 ug/mL and 101 ug/mL. No medical histories were available for the donors used in this study.

sensitivity

The minimum detectable dose of human Hemopexin is 12.5 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, three samples were spiked with high concentrations of Hemopexin in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:25)

		Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	102	98
	Range(%)	100-108	92-113
1:4	Average% of Expected	98	95
	Range(%)	77-120	81-114
1:8	Average% of Expected	100	96
	Range(%)	96-105	84-109
1:16	Average% of Expected	-	94
	Range(%)	-	87-99

references

1. Tolosano E. et al. (2002). DNA Cell Biol. 21(4):297-306. (PMID: 12042069)

2. Mehta NU. et al. (2015). Curr Opin Lipidol. 26(5): 384-7.(PMID: 26339767)

3. BrLin T. et al. (2015). Crit Care. 19:166.(PMID: 25888135)