Human ENPP-2/Autotaxin Sandwich ELISA Kit Datasheet

For the quantitative detection of human ENPP-2/Autotaxin concentrations in serum and plasma.

General Information

Catalogue Number	KE00179
Product Name	Human ENPP-2/Autotaxin Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	0.625-40 ng/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	5168
SwissProt	Q13822

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 80 ng/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 μL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle. For Human serum and plasma	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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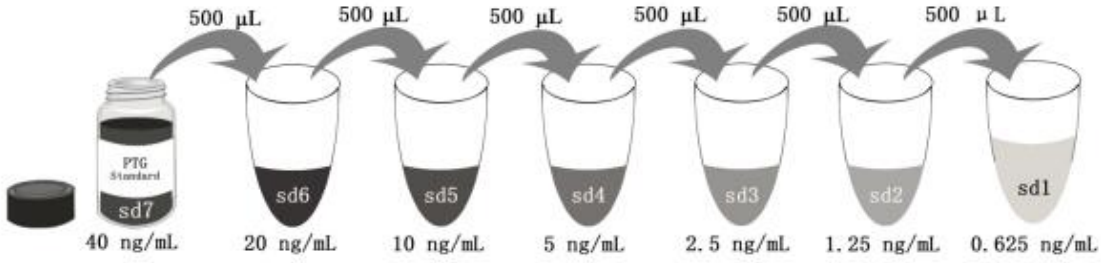
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ef is for protein standard, serum and plasma.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **2 mL** Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 40 ng/mL.



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	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00179 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The ENPP-2/Autotaxin ELISA kit is to be used to detect and quantify protein levels of endogenous ENPP-2/Autotaxin. The assay recognizes human ENPP-2/Autotaxin. An antibody specific for ENPP-2/Autotaxin has been pre-coated onto the microwells. The ENPP-2/Autotaxin protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human ENPP-2/Autotaxin is added to detect the captured human ENPP-2/Autotaxin 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 450 nm with the correction wavelength set at 630 nm.

Background

ENPP2, Autotaxin or Extracellular lysophospholipase D, is a 863 amino acid secreted protein, which is detected in blood plasma (at protein level) (PubMed:12176993, PubMed:26371182). ENPP2 is Predominantly expressed in brain, placenta, ovary, and small intestine. ENPP2 is expressed in a number of carcinomas such as hepatocellular and prostate carcinoma, neuroblastoma and non-small-cell lung cancer. ENPP2 is expressed in body fluids such as plasma, cerebral spinal fluid (CSF), saliva, follicular and amniotic fluids. Biliary atresia patients had greater serum autotaxin and liver stiffness than controls. Serum autotaxin and liver stiffness were markedly elevated in Biliary atresia patients with jaundice compared to those without jaundice. Furthermore, serum autotaxin was correlated with liver stiffness and biochemical parameters in Biliary atresia. This kit is used to quality ENPP2 level in serum.

Sample Preparation

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Different samples may require proper dilution to fall within the range of the assay. The serum or plasma is better to be diluted 1:50 or 1:100 before assay.

Safety Notes

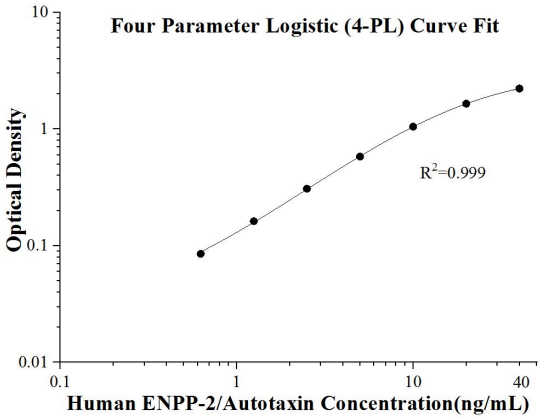
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.



(ng/mL)	O.D	Average	Corrected
0	0.062 0.062	0.062	-
0.625	0.144 0.149	0.147	0.085
1.25	0.222 0.225	0.224	0.162
2.5	0.371 0.368	0.370	0.308
5	0.642 0.642	0.642	0.58
10	1.091 1.127	1.109	1.047
20	1.707 1.711	1.709	1.647
40	2.287 2.285	2.286	2.224

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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				
Sample	n	Mean (ng/mL)	SD	CV%
1	20	2.53	0.09	3.5
2	20	5.95	0.19	3.1
3	20	19.19	0.30	1.6

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	2.35	0.11	4.6
2	24	5.66	0.17	3.0
3	24	18.53	0.48	2.6

Recovery

The recovery of ENPP-2/Autotaxin 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	1:200	93	70-110
	1:400	93	75-103

Sample Values

Serum, plasma samples from healthy volunteers were evaluated for ENPP-2/Autotaxin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Human serum (n=40)	395.72	53.10-1162.63

Sensitivity

The minimum detectable dose of human ENPP-2/Autotaxin is 0.01 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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Linearity

To assess the linearity of the assay, serum and plasma 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 1:25)

		Human serum
1:2	Average% of Expected	100
	Range (%)	-
1:4	Average% of Expected	91
	Range (%)	88-94
1:8	Average% of Expected	81
	Range (%)	79-83
1:16	Average% of Expected	78
	Range (%)	71-82

References

1. Tokumura A. et. al. (2002) J Biol Chem.18;277(42):39436-42.
2. Stein AJ et. al. (2015) Mol Pharmacol;88(6):982-92.
3. Udomsinprasert W et. al. (2015) Biomarkers. ;20(1):89-94.

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