Human EGF Sandwich ELISA Kit Datasheet

For the quantitative detection of Human EGF concentrations in serum, plasma, cell culture supernatants, urine and saliva.

General Information

Catalogue Number	KE00138
Product Name	Human EGF Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	125-8000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	1950
SwissProt	P01133

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 - 16000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 μL/vial	1 vial	
HRP-conjugated antibody (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 1-ef - 30 mL/bottle	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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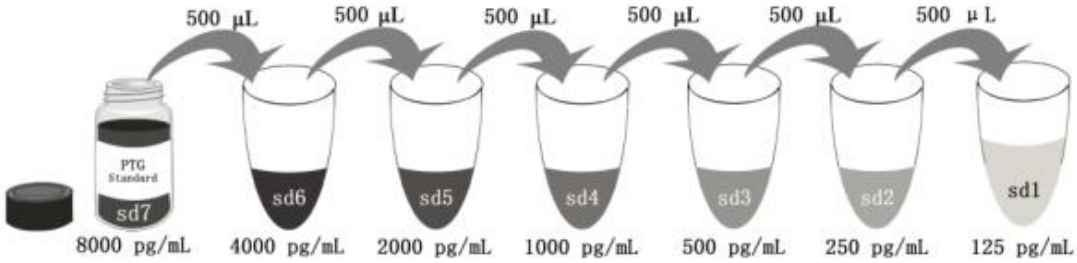
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1-ef is for protein standard and samples.
Detection Diluent is for Detection antibody and HRP-conjugated antibody.
*Add **2 mL** Sample Diluent PT 1-ef in protein standard. This reconstitution gives a stock solution of 8000 pg/mL.



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

Product Description

KE00138 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The EGF ELISA kit is to be used to detect and quantify protein levels of endogenous EGF. The assay recognizes human EGF. An antibody specific for EGF has been pre-coated onto the microwells. The EGF protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for EGF is added to detect the captured EGF 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

Epidermal growth factor (EGF) is a member of the epidermal growth factor superfamily. EGF preproprotein is proteolytically processed to generate the 53-amino acid epidermal growth factor peptide. EGF binds to the EGF receptor on the surface of cells and mediates intrinsic phosphorylation of the receptor on tyrosine residues. It has been detected in nearly all body fluids, such as urine (urogastrone), saliva, milk and platelet-rich plasma. EGF plays important roles in multiple biological processes, such as the regulation of cell growth, proliferation, and differentiation.

Sample Preparation

The samples may require proper dilution to fall within the range of the assay. The serum, plasma, cell culture supernatants and saliva is bettle to be diluented 1:2 or 1:4 before assasy and 1:16 or 1:32 dilution is recommended for urine.

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Safety Notes

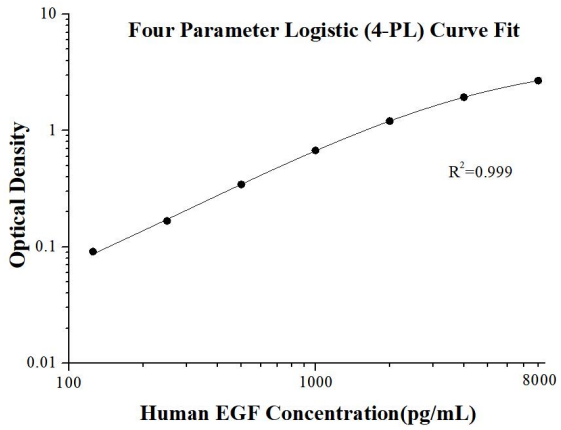
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.				

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	0.062 0.061	0.062	-
125	0.155 0.150	0.153	0.091
250	0.232 0.225	0.229	0.167
500	0.409 0.402	0.406	0.344
1000	0.709 0.762	0.736	0.674
2000	1.338 1.200	1.269	1.208
4000	2.082 1.910	1.996	1.935
8000	2.799 2.700	2.750	2.688

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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 (pg/mL)	SD	CV%
1	20	531.9	18.2	3.4
2	20	2,019.4	63.1	3.1
3	20	7,679.1	273.4	3.6

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	583.4	37.5	6.4
2	24	2,306.4	182.9	7.9
3	24	7,538.8	608.2	8.1

Recovery

The recovery of EGF 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 plasma	1:2	88	75-110
	1:4	88	71-104
Human serum	1:2	87	71-101
	1:4	97	82-112
Cell culture supernatants	1:2	105	88-123
	1:4	97	80-115
Saliva	1:2	102	99-105
	1:4	102	79-127
Urine	1:16	106	100-123
	1:32	109	90-125

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Sample Values

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

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Human serum (n=24)	1,220	371-2,701
Urine (n=7)	20,104	5,977-37,223
Saliva (n=7)	3,378	1,306-5,281

Sensitivity

The minimum detectable dose of human EGF is 26.9 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, the plasma samples and cell culture supernatants were spiked with high concentrations of EGF in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Serum, saliva and urine samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The urine sample were initially diluted 1:8)

		Human plasma	Human serum	Cell culture supernatants	Saliva	Urine
1:2	Average% of Expected	96	100	109	100	100
	Range (%)	93-100	-	96-123	-	-
1:4	Average% of Expected	106	95	106	100	101
	Range (%)	100-111	92-101	98-114	99-101	96-118
1:8	Average% of Expected	111	92	114	94	107
	Range (%)	102-120	85-104	101-128	89-104	104-116
1:16	Average% of Expected	118	-	120	-	-
	Range (%)	114-121	-	116-124	-	-

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References

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