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Antibodies | ELISA kits | Proteins

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human EGFR in serum, plasma, cell culture supernatants, cell lysates and human milk

general information

Catalogue Number	KE00142
Product Name	EGFR ELISA Kit
Species cross-reactivity	Human EGFR
Range (calibration Range)	46.88 – 3000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**1956** (Human)
SwissProt	**P00533** (Human)

kit components & storage

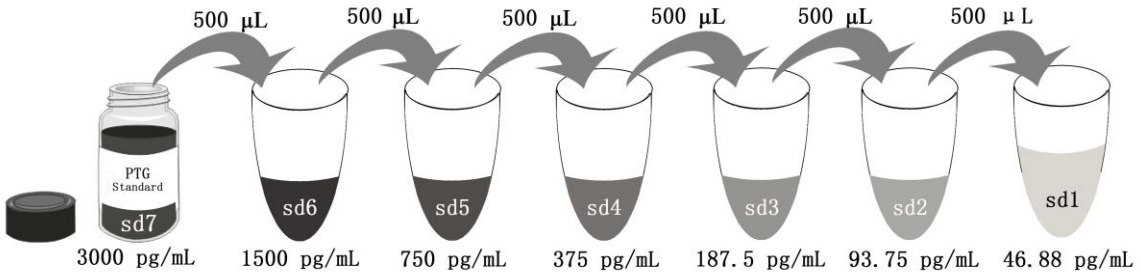
Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard - 3000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody, HRP-conjugated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1 - 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
Extraction Reagent - 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 is for standard and samples

Detection Diluent is for Detection antibody.

*Add **1 mL** Sample Diluent PT 1 in Standard. This reconstitution gives a stock solution of 3000 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	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00142 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The EGFR ELISA kit is to be used to detect and quantify protein levels of endogenous EGFR. The assay recognizes human EGFR. An antibody specific for EGFR has been pre-coated onto the microwells. The EGFR protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for EGFR is added to detect the captured EGFR 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 450nm with the correction wavelength set at 630 nm .

background

The epidermal growth factor receptor (EGFR) belongs to the ErbB family of receptor tyrosine kinases (RTKs) and exerts critical functions in epithelial cell physiology. EGFR is a receptor of tyrosine kinase (RTK). It is consisted of a C-terminus intracellular region that possesses the kinase activity, and an N-terminus extracellular ligand-binding site, a hydrophobic transmembrane domain. EGFR is frequently mutated and/or overexpressed in different types of human cancers and is the target of multiple cancer therapies currently adopted in the clinical practice. Mutations in this gene are associated with lung cancer.

sample preparation

The serum or plasma, milk, cell lysates, cell culture supernatants may require proper dilution to fall within the range of the assay. 1:100 or 1:200 dilution is recommended for healthy human serum or plasma. 1:2 dilution is recommended for milk. 1:128 or 1:256 dilution is recommended for Hela and HepG2 cell lysates. 1:2 dilution is recommended for cell culture supernatants.

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safety notes

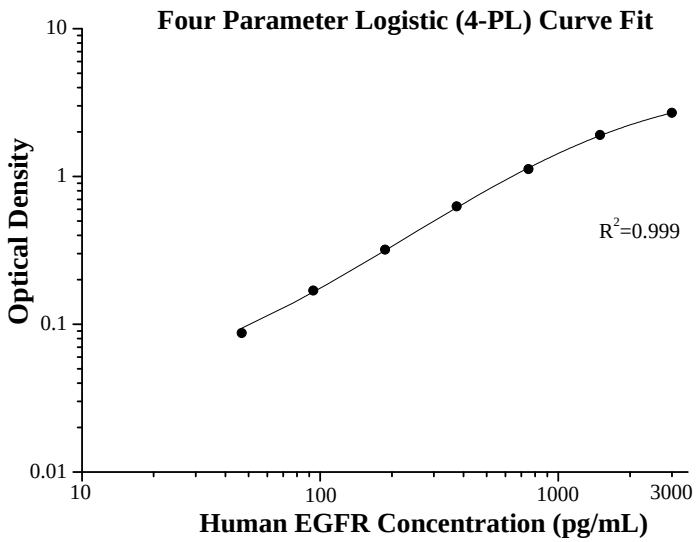
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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 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°C
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.



(pg/mL)	O.D	Average	Corrected
0	0.025 0.025	0.025	—
46.88	0.111 0.113	0.112	0.087
93.75	0.192 0.195	0.194	0.169
187.5	0.34 0.35	0.345	0.32
375	0.656 0.647	0.652	0.627
750	1.128 1.165	1.147	1.122
1500	1.901 1.956	1.929	1.904
3000	2.708 2.72	2.714	2.689

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.

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	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	1,265.9	322.7	79.9	1,249.3	313.6	76.2
SD	19.6	9.5	1.7	94.5	20.2	6.6
CV%	1.6	2.9	2.1	7.6	6.5	8.6

recovery

The recovery of EGFR 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	1:100	94	74-107
	1:200	98	83-111
Human milk	1:2	104	94-112
	1:4	99	91-113
Cell lysates	1:256	97	81-108
	1:512	96	83-114
Cell culture supernatants	1:2	89	76-97
	1:4	88	78-95

sample values

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

Sample Type	Mean(ng/mL)	Rang (ng/mL)
Human serum (n=24)	44.1	5.9-80.5
Human milk (n=8)	0.4	0.2-1.7

cell culture lysates:

Sample Type	EGFR (ng/mL)	Total protein (mg/mL)
Hela	53.9	3
HepG2	163.1	3

sensitivity

The minimum detectable dose of human EGFR is 0.1 pg/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, human serum, milk, cell lysates samples 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, the cell lysates samples were initially diluted 1:64)

To assess the linearity of the assay, cell culture supernatants were spiked with high concentrations of EGFR in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human serum	Human milk	Cell lysates	Cell culture supernatants
1:2	Average% of Expected	100	100	100	97
	Range (%)	-	-	-	95-100
1:4	Average% of Expected	99	105	105	94
	Range (%)	94-101	103-108	102-107	92-96
1:8	Average% of Expected	99	113	108	98
	Range (%)	94-104	107-119	103-113	96-100
1:16	Average% of Expected	101	119	116	99
	Range (%)	96-108	110-128	111-121	97-101

References

1. Schlessinger J. et al. (2014). Cold Spring Harb Perspect Biol. 6(3).
2. Yoshida T. et al. (2010). Biochem Pharmacol. 80(5):613-23.
3. Wilson KJ. et al. (2012). Growth Factors. 30(2):107-16.
4. Sigismund S. et al. (2018). Mol Oncol. 12(1):3-20.

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