

Product datasheet

Human soluble EGFR ELISA Kit **ab193764**
SimpleStep ELISA[®]

1 References 6 Images

Overview

Product name Human soluble EGFR ELISA Kit
Detection method Colorimetric
Precision

Intra-assay

Sample	n	Mean	SD	CV%
Human serum	5			2%

Inter-assay

Sample	n	Mean	SD	CV%
Human serum	3			3%

Sample type Cell culture supernatant, Serum, Cell culture extracts, Hep Plasma, EDTA Plasma, Cit plasma
Assay type Sandwich (quantitative)
Sensitivity 1 pg/ml
Range 78.125 pg/ml - 5000 pg/ml
Recovery

Sample specific recovery

Sample type	Average %	Range
Serum	95.2	89.1% - 98.9%
Cell culture media	111	110.3% - 111.8%
Hep Plasma	85.8	82.6% - 88.4%
EDTA Plasma	80.5	65.5% - 88.4%
Cit plasma	83.1	73.8% - 89.3%
Goat Serum	80	78.3% - 80.9%

Assay time	1h 30m
Assay duration	One step assay
Species reactivity	Reacts with: Human
Product overview	Human soluble EGFR ELISA kit has been re-developed. We have identified new recombinant monoclonal antibodies to provide improved performance and consistency. This version will be discontinued when inventory is depleted. The new version is available as ab269558

Human soluble EGFR ELISA Kit (ab193764) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of soluble EGFR protein in cell culture extracts, cell culture supernatant, cit plasma, edta plasma, hep plasma, and serum. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human soluble EGFR with 1 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate ([ab203359](#)) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

Notes	<p>EGFR is a receptor tyrosine kinase binding ligands of the EGF family and activating several signaling cascades to convert extracellular cues into appropriate cellular responses. The known ligands include EGF, TGFA/TGF-alpha, amphiregulin, epigen/EPGN, BTC/betacellulin, epiregulin/EREG and HBEGF/heparin-binding EGF. The ligand binding triggers receptor homo- and/or heterodimerization and autophosphorylation on key cytoplasmic residues. The phosphorylated receptor recruits adapter proteins like GRB2 which in turn activates complex downstream signaling cascades. EGFR activates at least 4 major downstream signaling cascades including the RAS-RAF-MEK-ERK, PI3 kinase-AKT, PLCgamma-PKC and STATs modules. It may also activate the NF-kappa-B signaling cascade. EGFR also directly phosphorylates other proteins like RGS16, activating its GTPase activity and probably coupling the EGF receptor signaling to the G protein-coupled receptor signaling. It also phosphorylates MUC1 and increases its interaction with SRC and CTNNB1/beta-catenin. Endocytosis and inhibition of the activated EGFR by phosphatases like PTPRJ and PTPRK constitute immediate regulatory mechanisms. Upon EGF-binding EPS15 is phosphorylated and it regulates EGFR endocytosis and activity. Moreover, inducible feedback inhibitors including LRIG1, SOCS4, SOCS5 and ERRF1 constitute alternative regulatory mechanisms for the EGFR signaling. EGFR also forms a heterodimer with ERBB2. EGFR is single-pass type I membrane protein of plasma membrane and many intracellular membranes. In response to EGF, EGFR is translocated from the cell membrane to the nucleus via Golgi and ER. EGFR undergo endocytosis upon activation by ligand. Isoform 2 is secreted. EGFR is ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.</p>
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It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform

Microplate (12 x 8 well strips)

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

Components	
Plate Seals	1 x unit
10X Wash Buffer PT (ab206977)	1 x 20ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
TMB Development Solution	1 x 12ml
Stop Solution	1 x 12ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 x unit
10X Human soluble EGFR Capture Antibody	1 x 600µl
10X Human soluble EGFR Detector Antibody	1 x 600µl
Human soluble EGFR Lyophilized Recombinant Protein	2 x 1vial
Sample Diluent NS (ab193972)	1 x 50ml
Antibody Diluent CPI - HAMA Blocker (ab193969)	1 x 6ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml

Function

Receptor tyrosine kinase binding ligands of the EGF family and activating several signaling cascades to convert extracellular cues into appropriate cellular responses. Known ligands include EGF, TGFA/TGF- α , amphiregulin, epigen/EPGN, BTC/betacellulin, epiregulin/EREG and HBEGF/heparin-binding EGF. Ligand binding triggers receptor homo- and/or heterodimerization and autophosphorylation on key cytoplasmic residues. The phosphorylated receptor recruits adapter proteins like GRB2 which in turn activates complex downstream signaling cascades. Activates at least 4 major downstream signaling cascades including the RAS-RAF-MEK-ERK, PI3 kinase-AKT, PLCgamma-PKC and STATs modules. May also activate the NF-kappa-B signaling cascade. Also directly phosphorylates other proteins like RGS16, activating its GTPase activity and probably coupling the EGF receptor signaling to the G protein-coupled receptor signaling. Also phosphorylates MUC1 and increases its interaction with SRC and CTNNB1/beta-catenin.

Isoform 2 may act as an antagonist of EGF action.

Tissue specificity

Ubiquitously expressed. Isoform 2 is also expressed in ovarian cancers.

Involvement in disease

Lung cancer

Inflammatory skin and bowel disease, neonatal, 2

Sequence similarities

Belongs to the protein kinase superfamily. Tyr protein kinase family. EGF receptor subfamily. Contains 1 protein kinase domain.

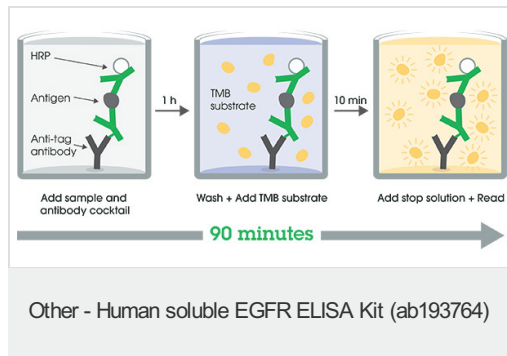
Post-translational modifications

Phosphorylation at Ser-695 is partial and occurs only if Thr-693 is phosphorylated. Phosphorylation at Thr-678 and Thr-693 by PRKD1 inhibits EGF-induced MAPK8/JNK1 activation. Dephosphorylation by PTPRJ prevents endocytosis and stabilizes the receptor at the plasma membrane. Autophosphorylation at Tyr-1197 is stimulated by methylation at Arg-1199 and enhances interaction with PTPN6. Autophosphorylation at Tyr-1092 and/or Tyr-1110 recruits STAT3. Dephosphorylated by PTPN1 and PTPN2. Monoubiquitinated and polyubiquitinated upon EGF stimulation; which does not affect tyrosine kinase activity or signaling capacity but may play a role in lysosomal targeting. Polyubiquitin linkage is mainly through 'Lys-63', but linkage through 'Lys-48', 'Lys-11' and 'Lys-29' also occurs. Deubiquitination by OTUD7B prevents degradation. Ubiquitinated by RNF115 and RNF126. Methylated. Methylation at Arg-1199 by PRMT5 stimulates phosphorylation at Tyr-1197.

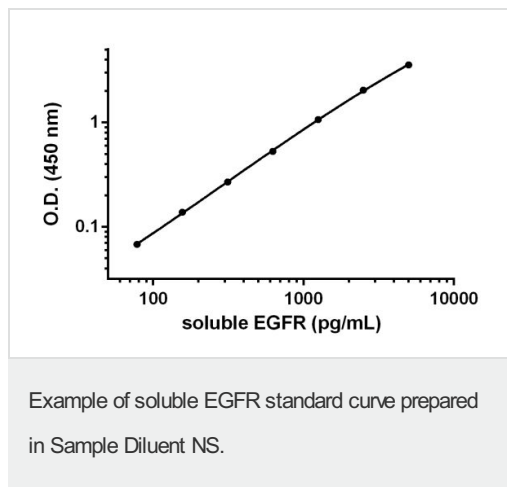
Cellular localization

Secreted and Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus membrane. Nucleus membrane. Endosome. Endosome membrane. Nucleus. In response to EGF, translocated from the cell membrane to the nucleus via Golgi and ER. Endocytosed upon activation by ligand. Colocalized with GPER1 in the nucleus of estrogen agonist-induced cancer-associated fibroblasts (CAF).

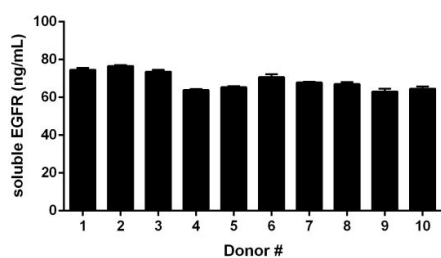
Images



SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.

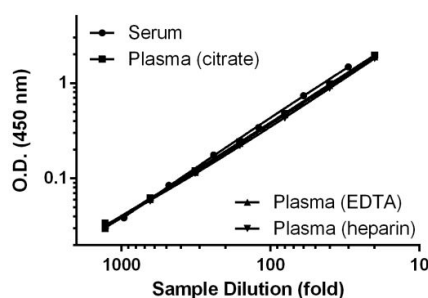


Background-subtracted data values (mean \pm SD) are graphed.



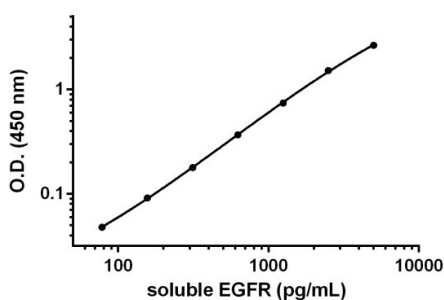
soluble EGFR concentrations in individual Human serum donors.

30X diluted serum samples from 10 apparently healthy male donors (1M – 10M) were measured in triplicates using this kit. Interpolated data values are graphed in ng of soluble EGFR per mL of serum (mean \pm SD, n=3). The mean of soluble EGFR concentration of these serum samples was determined to be 68.8 ng/mL with a range of 63.1 – 76.7 ng/mL.



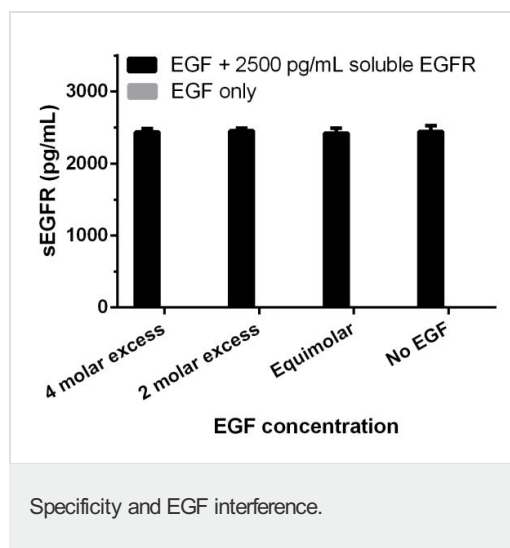
Titration of Human serum and plasma samples within the working range of the assay.

Background-subtracted data values (mean \pm SD, n = 2) are graphed.



Example of soluble EGFR standard curve prepared in 1X Cell Extraction Buffer PTR.

Background-subtracted data values (mean \pm SD) are graphed.



4 molar excess, 2 molar excess and equimolar concentrations of Human recombinant EGF (relatively to molarity corresponding to used soluble EGFR), as well as no EGF were tested in the presence of 2,500 pg/mL Human recombinant soluble EGFR. Same concentrations of EGF were tested also in the absence of soluble EGFR. Interpolated sample values (mean \pm SD, $n = 2$) are graphed. Note that in the tested range the EGF did not interfere with the soluble EGFR measurement. Also note that the EGF alone did not showed any significant cross-reactivity.

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