REFERENCES

Daly, R.J. (1999) Growth Factors, 16:255.

Schlessinger, J. (2000) Cell. 103:211.

Maihle, N.J. et al. (2002) Cancer Treat. Res. 107:247.

Human EGF R Immunoassay

Catalog Number: SEKH-0154

For the quantitative determination of human EGFR concentrations in cell culture supernates,serum, and plasma.

For research use only. Not for use in diagnostic procedures.

Country | Company: China | Beijing Solarbio Science & Technology Co.,Ltd
Address:NO.85A, Liandong U Valley, Tongzhou District, Beijing, P.R.China.
Tel: 86-10-56371241 Fax: 86-10-56371282 E-mail: service@solarbio.com

TABLE OF CONTENTS

LINEARITY: To assess the linearity of the assay, three samples 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.

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	88	101
	Range(%)	85-96	95-109
1:4	Average% of Expected	97	107
	Range(%)	91-104	102-116

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 7.8pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant human EGFR. The factors listed below were prepared at 200ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant porcine
Amphiregulin	EGF	
Betacellulin		
EGF		
Epiregulin		
ErbB2		
ErbB3		
ErbB4		
HB-EGF		
TGF-β1		
TGF-α		

REPEATABILITY: Factors assayed for cross-reactivity

RECOVERY: The recovery of EGFR spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of EGFR in two matrices

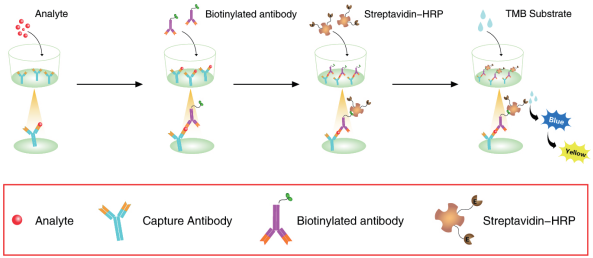
Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	96	90-105
Cell culture supernatants	104	98-112

BACKGROUND

The EGF R subfamily of receptor tyrosine kinases comprises four members: EGF R (also known as HER-1, ErbB1, or ErbB), ErbB2 (Neu, HER-2), ErbB3 (HER-3), and ErbB4 (HER-4). All family members are type I transmembrane glycoproteins with an extracellular ligand binding domain containing two cysteine-rich domains separated by a spacer region and a cytoplasmic domain containing a membrane-proximal tyrosine kinase domain followed by multiple tyrosine autophosphorylation sites. The human EGF R cDNA encodes a 1210 amino acid (aa) precursor with a 24 aa signal peptide, a 621 aa extracellular domain (ECD), a 23 aa transmembrane segment, and a 542 aa cytoplasmic domain. Soluble receptors consisting of the extracellular ligand binding domain are generated by alternate splicing in human and mouse. EGF R binds a subset of the EGF family ligands, including EGF, amphiregulin, TGF-alpha, betacellulin, epiregulin, HB-EGF, and epigen. EGF R signaling regulates multiple biological functions including cell proliferation, differentiation, motility, and apoptosis. EGF R is overexpressed in a wide variety of tumors and is the target of several anti-cancer drugs.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for EGFR has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any EGFR present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for EGFR is added to detect the captured EGFR protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. 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.



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

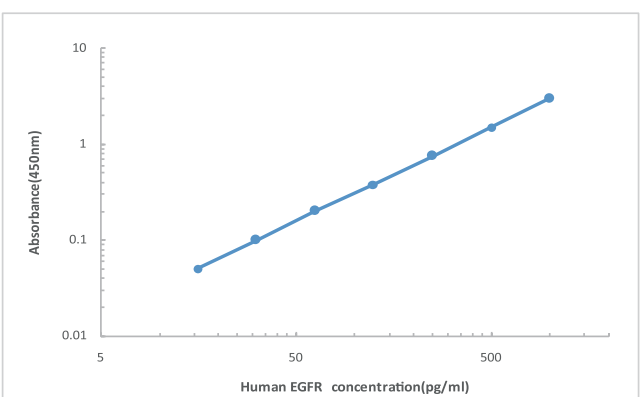
PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the EGFR ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.048	0.056	0.052	-
15.62	0.211	0.215	0.213	0.161
31.25	0.306	0.312	0.309	0.257
62.5	0.456	0.464	0.460	0.408
125	0.724	0.737	0.730	0.679
250	1.175	1.197	1.186	1.135
500	1.914	1.949	1.931	1.880
1000	3.118	3.176	3.147	3.095



Representative standard curve for EGFR ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed.

⇩

Add 100µl standard or samples to each well, incubate 90 minutes,37℃.

⇩ Aspirate and wash 4

Add 100µl working solution of Biotin-Conjugate anti-human EGFR antibody to each well, incubate 60 minutes,37℃.

⇩ Aspirate and wash 4

Add 100µl working solution of Streptavidin-HRP to each well, incubate 30 minutes,37℃.

⇩ Aspirate and wash 5

Add 100µl Substrate solution to each well, incubate 15 minutes,37℃. Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 30 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the EGFR concentrations versus the log of the O.D. and the best fit line can be determined by

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,2000 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.
6.Human Leptin controls (optional; available from Solarbio).

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge at approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

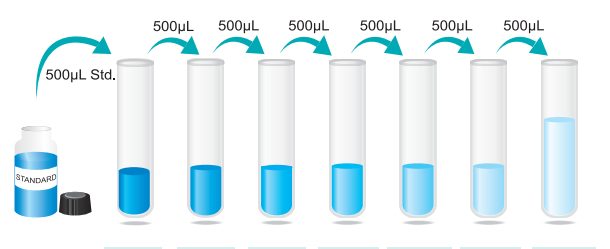
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning -** Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer -** Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard\Specimen -** Reconstitute the Standard with 1.0mL of deionized or distilled water. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500µL of Standard/Specimen Diluent into the 1000 pg/mL tube, and add 500µL stock solution of 2000 pg/mL into it to get the high standard of 1000 pg/mL. Pipette 500µL of Standard/Specimen Diluent into the remaining tubes. Use the high standard to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 1000 pg/mL standard serves as the high standard. The Standard/specimen Diluent serves as the zero standard (0 pg/mL).
   ***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**
4. **Working solution of Biotin-Conjugate anti-human EGFR antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**
5. **Working solution of Streptavidin-HRP:** Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**



Preparation of EGFR standard dilutions