HUMAN SOLUBLE EPIDERMAL GROWTH FACTOR RECEPTOR (sEGFR)/HER1 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE EGFR/HER1 CONCENTRATIONS IN CELL CULTURE SUPERNATES OR TISSUE HOMOGENATES, SERUM, AND EDTA PLASMA

PRODUCT INFORMATION:
THIS IS FOR ONE TIME USE ONLY

ELISA NAME	HUMAN SOLUBLE EGFR ELISA
Catalog No.	SK00469-06
Lot No.	
Formulation	96 T
Standard Range	7.8 - 500 pg/mL
Sensitivity	5 pg/mL
Sample Volume	100 µL
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates or Tissue Homogenates
Specificity	Human soluble EGFR/HER1
Calibration	Human soluble EGFR/HER1 recombinant
Dilution Factor	100 for serum or plasma **(Optimal dilutions should be determined by each laboratory for each application)**
Intra-assay Precision	6 - 8%
Inter-assay Precision	8 - 10%
Storage	2 – 8°C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Website: www.AvisceraBioscience.com

INTRODUCTION

EGF receptor is a type I transmembrane glycoprotein with an extracellular domain (ECD) that has two cysteine-rich regions. EGFR belongs to receptor tyrosine kinases superfamily. EGFR as a cell surface receptor that binds EGF, TGF-alpha, HB-EGF, Amphiregulin, Epigen/EPGN, BTC/Betacellulin and Epiregulin/EREG and triggers receptor homo- and/or heterodimerization and autophosphorylation. The levels of soluble EGFR are elevated in the serum of gastric carcinoma patients. However, the serum sEGFR are reduced in small cell lung cancer patients and other carcinoma patients.

DESCRIPTION

This Human Soluble Epidermal Growth Factor Receptor (sEGFR)/HER1 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human sEGFR from cell culture supernates or tissue homogenates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human sEGFR and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural sEGFR samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human sEGFR. The capture antibody can bind to the human sEGFR in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human sEGFR is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human sEGFR bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
sEGFR Microplate - 96 well microplate coated with an antibody against sEGFR.	**469-06-01**	**1 plate**
sEGFR Standard – refer to lot specific of lyophilized recombinant human sEGFR.	**469-06-02**	**1 vial**
Detection Antibody Concentrate – refer to lotspecific of 10-fold concentrate of lyophilized biotinylated antibody against sEGFR.	**469-06-03**	**1 vial**
Positive Control - one vial of lyophilized recombinant human sEGFR.	**469-06-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 40 mL of buffered protein based solution with preservative.	**DB01**	**2 bottles**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08A**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**

Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 10 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20°C or -70°C.
For Longer storage for Dilution Buffer (**DB01**), store at -20°C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 ml and 500 ml graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and 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 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and 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 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) (Aviscera Order Code: 00740-01-25, 25 TIU) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may require a 100-fold or 200-fold dilution. A suggested 100-fold dilution is 5 µl sample + 495 µl Dilution Buffer. A suggested 200-fold dilution is 5 µl sample + 995 µl Dilution Buffer.
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 ml of Wash Buffer Concentrate into deionized or distilled water (450 ml) to prepare 500 ml of 1x Wash Buffer.

sEGFR Standard - Reconstitute the sEGFR standard with refer to lot specifc of Dilution Buffer. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µl of Dilution Buffer into tubes #2 to #7. Use the stock solution to produce a dilution series (next page). Mix each tube thoroughly before the next transfer. The **500 pg/ml** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/ml).

Tube	Standard	Dilution Buffer	Concentration
Stock	Powder	Lot specific	
# 1	Lot specific	Lot specific	500 pg/ml
# 2	250 µl of 1	250 µl	250 pg/ml
# 3	250 µl of 2	250 µl	125 pg/ml
# 4	250 µl of 3	250 µl	62.5 pg/ml
# 5	250 µl of 4	250 µl	31.25 pg/ml
# 6	250 µl of 5	250 µl	15.6 pg/ml
# 7	250 µl of 6	250 µl	7.8 pg/ml

Positive Control - Reconstitute the Positive Control with refer to lot specific of Dilution Buffer.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with lot specific of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 ml of Dilution Buffer into a 15 ml centrifuge tube and transfer 1.05 ml of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 ml of **HRP Diluent Solution (DB08a)** into a 15 ml centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution. **(protect from light)**.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot specific minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Samples with a concentration reading exceeding that of standard 500 pg/ml may result in inaccurate, low human sEGFR levels. Such samples require further external predilution according to expected human sEGFR values with Dilution Buffer in order to precisely quantify the actual human sEGFR level.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human sEGFR	100
Human HER2	0
Human HER3	0
Human HER4	0

TYPICAL STANDARD CURVE

This standard curve is for demonstration only. A new standard curve should be made for each set of samples assayed.

sEGFR (pg/mL)	Average OD450 (Corrected)
Blank	Refer to lot
7.813	0.054
15.625	0.098
31.25	0.211
62.5	0.401
125	0.774
250	1.479
500	2.566

LINEARITY

To assess the linearity of the assay, pooled research human **serum** samples were diluted with Dilution Buffer (DB01) and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (NG/ML)	RECOVERY (%)
50	732.665	36.633	100
100	395.784	39.578	108
200	204.843	40.969	112

To assess the linearity of the assay, pooled research human **EDTA plasma** samples were diluted with Dilution Buffer (DB01) and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (NG/ML)	RECOVERY (%)
50	936.316	46.816	100
100	481.387	48.139	103
200	235.870	47.174	101

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate refer to lot specific min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 15 min.