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

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

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN SOLUBLE EGFR ELISA
Catalog No.	SK00469-01
Lot No.	
Formulation	96 T
Standard range	15.6-1000 pg/mL
Sensitivity	5 pg/mL
Sample Volume	100 µl
Sample Type	Serum, Plasma, Cell Culture, Tissue Homogenates
Dilution Factor	100 for serum or plasma (Optimal dilutions should be determined by each laboratory for each application)
Specificity	Human soluble EGFR/Her1
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 - 8°C

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INTRODUCTION

EGF receptor is a type I transmembrane glycoprotein with an extracellular domain (ECD) that has two cycteine-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.

Human Soluble Epidermal Growth factor Receptor (sEGFR)/ immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human sEGFR in cell culture supernates, serum, and EDTA plasma. It contains recombinant human sEGFR and antibodies raised against this protein. It has been shown to accurately quantify recombinant human sEGFR. Results obtained with naturally occurring sEGFR samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human sEGFR.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for sEGFR has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sEGFR present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for sEGFR is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of sEGFR bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute 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.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
sEGFR Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against sEGFR.	**469-01-01**	**1 plate**
sEGFR Standard – 1000 pg/vial of recombinant human sEGFR in a buffered protein base with preservatives; lyophilized.	**469-01-02**	**1 vial**
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of biotinylated antibody against sEGFR with preservatives; lyophilized.	**469-01-03**	**1 vial**
Positive Control - one vial of recombinant human sEGFR, lyophilized	**469-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB01**	**1 bottle**
Antibody Diluent Solution Concentrate – 11 mL of buffered protein based solution with preservatives	**DB20**	**1 tube**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate	**TMB01**	**1 bottle**

solution		
Stop Solution - 11 mL of 0.5M HCl solution	**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 12 months. For longer storage, unopened Standard, Positive Control, Detection Antibody Concentrate and Antibody Diluent Solution Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard, Detection Antibody Concentrate Solution and Antibody Diluent Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated and other components may be stored at 2 - 8°C for up to 12 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8°C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

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 - 70° 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 -70° C. Avoid repeated freeze-thaw cycles.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may require an 100-fold dilution. A suggested 100-fold dilution is 10 µL sample + 90 µL Dilution Buffer. Following 30 µL 10-fold diluted sample + 270 µL Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

REAGENT PREPARATION

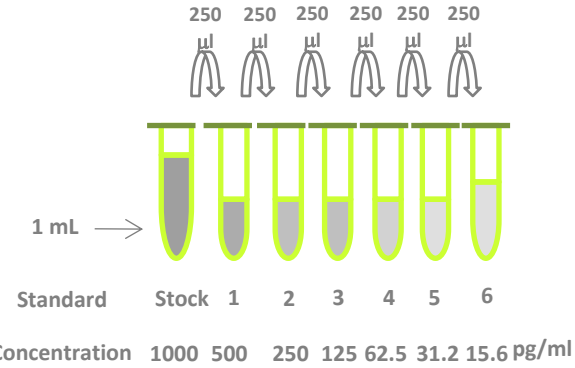
Bring all reagents to room temperature before use.

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 Wash Buffer.

Antibody Diluent Solution Concentrate – Reconstitute the Antibody Diluent Solution Concentrate with 11.0 mL of Dilution Buffer in provided 15 mL centrifuge tube to prepare Antibody Diluent Solution.

sEGFR Standard - Refer to vial label for reconstitution volume. Reconstitute the **sEGFR** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 1000 pg/mL. 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 #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 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	1.0 ml	1000 pg/ml
# 1	250µl of stock	250µl	500 pg/ml
# 2	250µl of 1	250µl	250 pg/ml
# 3	250µl of 2	250µl	125 pg/ml
# 4	250µl of 3	250µl	62.5 pg/ml
# 5	250µl of 4	250µl	31.25 pg/ml
# 6	250µl of 5	250µl	15.6 pg/ml



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 105 µL of Antibody Diluent Solution to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Antibody Diluent Solution into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution. **Note: Prepare 1-2 hours prior to use.**

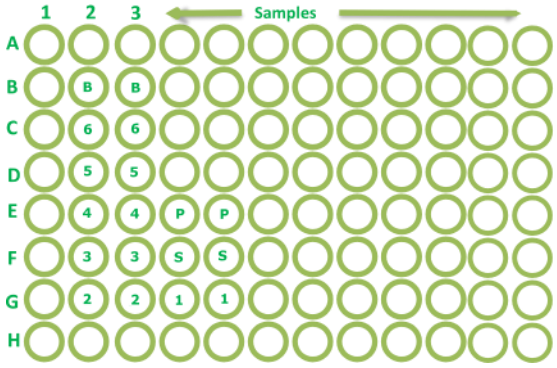
Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used within a few days.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of Standard (from C2, C3 to G2, G3 and F4, F5 to G4, G5), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed. **Note:** Mark strips #1-12 for your own reference in case strips fall out of plate frame.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 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 micro-plate 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 micro-plate 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 5-10 minutes 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 micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log 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. The data may be linearized by plotting the log of the sEGFR concentrations versus the log of the O.D. and the best fit line can be determined by 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.

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	**0 (0.082)**
15.6	**0.032**
31.25	**0.069**
62.5	**0.137**
125	**0.221**
250	**0.462**
500	**0.938**
1000	**1.880**

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human EGF Receptor, extracellular domain.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of sEGFR was 5 pg/mL.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human sEGFR	**100**
Human Her2	**0**
Human Her3	**0**
Human Her4	**0**

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl of standard, samples, positive control to each 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 5-10 min on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450 nm within 15 min