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

INTRODUCTION

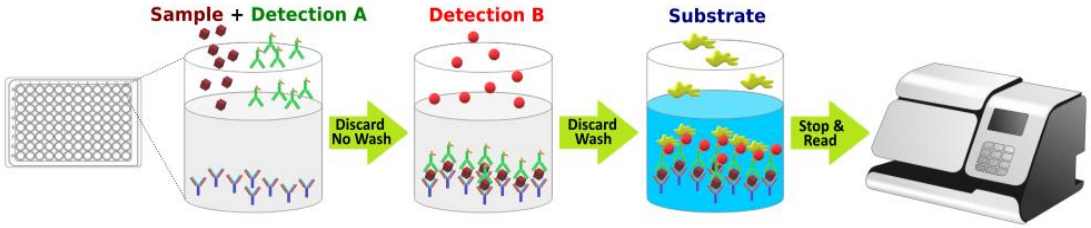
EGFR is a type I transmembrane glycoprotein that has an extracellular structural domain (ECD) containing two cysteine-rich regions separated by a spacer region. Its cytoplasmic structural domain contains a proximal membrane tyrosine kinase structural domain followed by multiple serine, threonine, and tyrosine phosphorylation sites. Adult EGFR is a 170 kDa protein extensively heterogeneously modified by N-linked glycosylation. EGFR is widely expressed on epithelial cells, primarily in the gastrointestinal tract and mammary gland, and is required for epithelial cell development and proliferation.

The Tribo™ Fast Human EGFR ELISA is designed to quantitatively detect Human EGFR levels in different tissues including skin, muscle, neural, serum, and other biological samples. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 3 hours, with no need for 4-5 hours (Fig. 1). The detection range is from 3 to 200 pg/mL. The levels of human EGFR samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural human EGFR protein.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative e sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for human EGFR was pre-coated onto a microplate. Standards and samples are pipetted into the wells, and then incubated with biotin-conjugated detection antibody specific for human EGFR. After washing, incubate the plate with HRP-Streptavidin. Following a wash to remove any unbound antibody and samples, an ultra-sensitive TMB substrate solution is added to the wells for color development. The color intensity is in proportion to the amount of EGFR bound in the initial step. The intensity of the color is measured by plate read at 450 nm.

Fig. 1: Assay Principle:



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Human EGFR Microplate	TBS3250A	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human EGFR.	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Human EGFR Standard	TBS3250B	15 µl of Recombinant human EGFR protein (20ng/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3250C	2.1 ml of Biotin-Human EGFR antibody.	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS3250D	300 µl of Streptavidin-HRP.	
Assay Diluent	TBS3250E	25 ml of a buffered protein base with preservatives.	
Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 2 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.).*

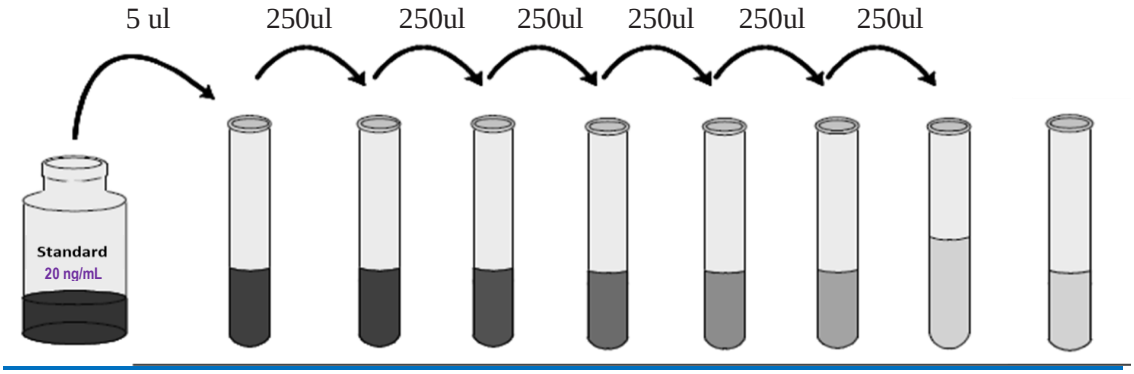
Detection B working solution preparation: Add 240 µL of **Detection B** streptavidin-HRP to 12 mL Assay Diluent to prepare Detrection B working solution.

Human EGFR Standard Preparation:

Label test tubes as #1 through #8. Pipet 495 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #7 **as diagram below (Fig.2).**

1. Add 5 µL of the Human EGFR Standard stock solution (20ng/mL) by dilution of 100X to tube #1 (200pg/mL), and mix.
2. Make 2x serial dilutions of the standard using the 200pg/mL standard solution (tube #1) from tube #2 through #7 with sequential transfer of 250 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 200, 100, 50, 25, 12.5, 6.25, and 3.13 pg/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for Human EGFR standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	
Assay Buffer (µL)	495	250	250	250	250	250	250	250
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	5	250	250	250	250	250	250	0
Final Conc (pg/ml)	200	100	50	25	12.5	6.25	3.13	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours**.
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B** to each well. Incubate at **RT for 1 hour.**
5. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer (*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 **TMB Substrate** to each well. Incubate **at RT for 10-20 min** (*Protect from light*). The color becomes blue.

7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
8. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve 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. The data may be linearized by plotting the log of the human EGFR 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.

TYPICAL DATA

This standard curve(R^2=0.9995) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

SENSITIVITY

The minimum detectable dose (MOD) of human EGFR is typically 2 pg/ml.
The Intra-assay CV is 3.79%, the Inter-assay CV is <10%.

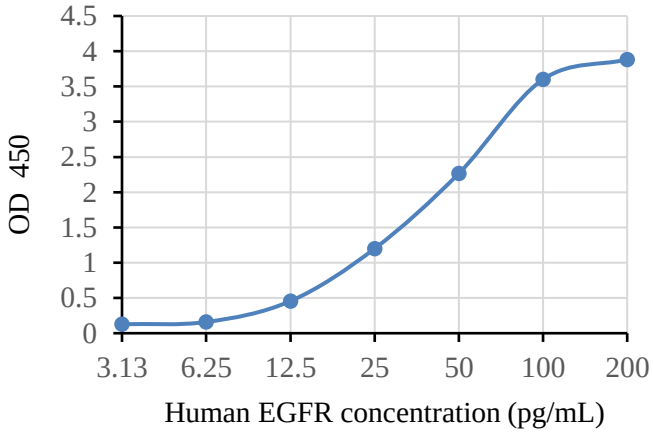
SPECIFICITY

This assay recognizes natural and recombinant human EGFR.

RELATIVE PRODUCTS

Human IL-1β ELISA (TBS3219)
Human IL-2 ELISA (TBS3220)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)

Fig.3: Human EGFR Standard Curve



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