Tribioscience

Fast EGFR Kinase Activity Assay (Catalog Number: TBS2048; 100 assays)

INTRODUCTION

The epidermal growth factor receptor (EGFR; ErbB-1; HER1) is a receptor tyrosine kinase critical for regulating cell proliferation, differentiation, survival, migration, and organ development. Dysregulation of EGFR signaling-through mutations, overexpression, or loss-of-function, is implicated in a diverse spectrum of human diseases.

Tribioscience's Fast EGFR Kinase Activity Assay is designed to quantitatively detect EGFR Kinase Activity levels in different tissues including skin, muscle, neural, serum, and other biological samples. In this assay EGFR phosphorates tyrosine-based substrate in the presence of co-substrate ATP. The phosphotyrosine concentration can be detected with anti-phorsphotyrosine based sandwich ELISA technology. The assay combines multiple reactions into a one-step. The measurement can be finished in 1.5 hours. The detection range is from 78 to 5000 pg/mL. The levels of sample EGFR Kinase activity 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 EGFR Kinase Activity.

Synonyms: ERBB, mENA, ERBB1, HER1

MAIN FEATURES

Simple: Multiple reactions into one-step.
Accurate: Directly measure the phorsphoration of tyrosine
Fast: Just 1.5 hours.

KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Substrate coated plate	TBS2048A	96 well polystyrene microplate (12 strips of 8 wells) coated with a specific substrate for EGFR kinase.	Return unused wells to the foil pouch. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.
Co-substrate	TBS2048B	50 µL of ATP (50x)	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
EGFR Standard	TBS2048C	20 µL of EGFR kinase (0.125 ng/µL).	
Activate Buffer	TBS2048D	6 mL of a buffer with activators.	
Detection A	TBS2048E	12 µL of anti-mouse phosphotyrosine antibody (200x).	
Detection B	TBS2048F	6 µL of anti-mouse IgG-HRP.	May be stored for up to 6 months at 2-8 °C.
Assay Diluent	TBS2048G	15 mL of a buffered protein base with preservatives.	
Wash Buffer	TBS2048H	12mL of concentrated solution (10x).	
TMB Substrate	TBS2048I	11 mL of ultra-sensitive TMB substrate.	
Stop Solution	TBS2048J	6mL of 2 N sulfuric acid.	

Store the unopened kit at -20 °C. 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 A and co-substrate working solution preparation: Add 10 µL of **Detection A (200x)** anti-mouse of phosphotyrosine antibody and 40µL of co-substrate into 1.95 mL Activator Buffer (TBS2048D) to prepare Detection A and co-substrate working solution.
Detection B working solution preparation: Add 5 µL of **Detection B** anti-mouse IgG -HRP to 10 mL Assay Diluent (TBS2048G) to prepare Detection B working solution.

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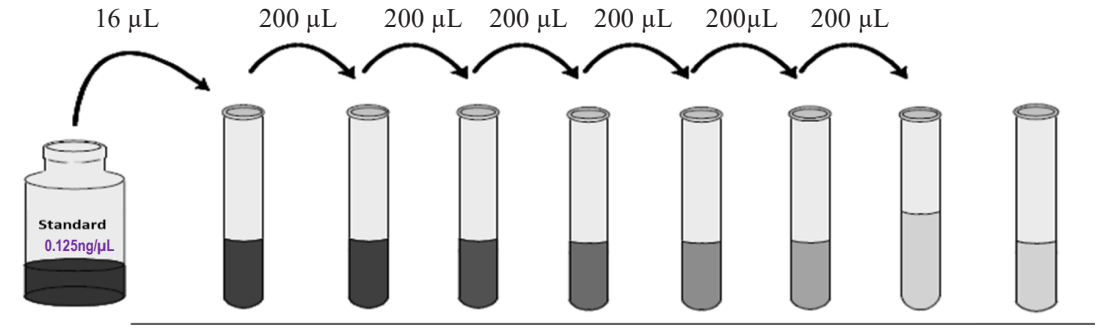
EGFR Standard Preparation:

Label test tubes as #1 through #8. Pipet 384 µL of 1x activate buffer into tube #1, and 200 µL into tubes #2 to #8 as Fig.1 diagram below.

1. Add 16 µL of EGFR Standard stock solution (25x, 0.125 ng/µL) by dilution of 25X to tube #1 and mix (5000 pg/mL).

2. Make 2x serial dilutions using the of 5000 pg/mL (tube #1) standard solution from tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 5000, 2500, 1250, 625, 312.5, 156.25, and 78.125 pg/mL. Tube# 8 is blank (0 pg/mL).

Fig.1 Diagram for EGFR Kinase standard preparation



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Activate Buffer (µL)	384	200	200	200	200	200	200	200
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	16	200	200	200	200	200	200	0
Final Conc (pg/ mL)	5000	2500	1250	625	312.5	156.25	78.125	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 and co-substrate working solution** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **37°C for 0.5 hours with shaking**.
3. Aspirate each well (no wash). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B working solution** to each well. Incubate at **RT for 0.5 hours with shaking.**
5. Aspirate each well and wash 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 minutes with shaking** (*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 be correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

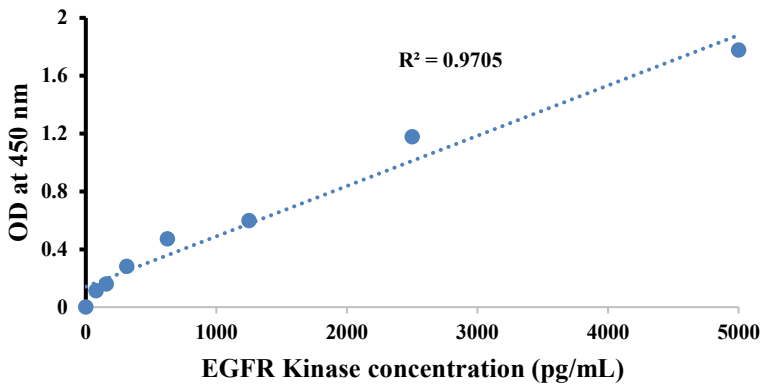
CALCULATION OF RESULTS

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 EGFR Kinase 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 is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 2 is an example of typical Data.

Fig.2: EGFR Kinase Activity Standard Curve



SENSITIVITY

The minimum detectable dose (MOD) of EGFR Kinase is typically 78 pg/ml.

Unit definition: 1 Unit (U) will catalyze the conversion of 1 µmole of substrate to phosphotyrosine per min at 37°C.

EGFR Activity (U/L) = DF * (ODSAMPLE – OD BLANK)/ (t * Slope)

where ODSAMPLE is the OD450nm value for each sample and ODBLANK is the OD450nm value of the sample blank. Slope is the slope of the linear regression fit of the standard points and t is the reaction time (30 min). DF is the dilution factor.

SPECIFICITY

This assay recognizes natural and recombinant EGFR Kinase.

RELATIVE PRODUCTS

Resazurin Cell Viability Kit (TBS2001)
ATP Colorimetric/Fluorometric Assay (TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
CCK-8 Cell Viability Assay (TBS2022)
Thiol Fluorometric Assay (TBS2026)
GSH Assay (TBS2028)
Caspase-3 Colorimetric Assay kit (TBS2030)
AHCY Activity Fluorometric Assay (TBS2056)
Glucose Oxidase Colorimetric/Fluorometric Assay (TBS2088)
Homocysteine Fluorometric Assay (TBS2091)
NNMT Inhibitor Screening Assay (TBS2097)
NNMT Activity Fluorometric Assay (TBS2098)
G6PDH Activity Colorimetric Assay (TBS2102)
AHCY Inhibitor Screening Fluorometric Assay (TBS2099)

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