Datasheet for ABIN1981716

EGFR ELISA Kit

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Overview

Quantity:	96 tests
Target:	EGFR
Binding Specificity:	pTyr
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Human Phosphotyrosine EGFR ELISA Kit. This assay semi-quantitatively measures phophorylated EGFR (Tyr1086) and Total EGFR in lysate samples.
Sample Type:	Cell Lysate, Tissue Lysate
Analytical Method:	Semi-Quantitative
Detection Method:	Colorimetric
Specificity:	The antibody pair provided in this kit recognizes Human Tyrosine-Phosphorylated-EGFR.
Characteristics:	• Rapidly measure phosphorylated protein in lysates • Screen numerous different cell lysates without performing a Western Blot analysis • Minimal hands-on time, convenient, and non-radioactive material
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Biotinylated Anti-Phosphotyrosine Antibody • Stop Solution

Product Details

	• Assay Diluent(s) • Positive Control Sample • Lysis Buffer • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • 100 mL and 1 liter graduated cylinders • Tubes to prepare sample dilutions • Protease and Phosphatase inhibitors • Precision pipettes to deliver 2 µL to 1 mL volumes • Adjustable 1-25 mL pipettes for reagent preparation • Benchtop rocker or shaker • Microplate reader capable of measuring absorbance at 450 nm

Target Details

Target:	EGFR
Alternative Name:	EGFR (EGFR Products)
Gene ID:	3236
UniProt:	P00533
Pathways:	NF-kappaB Signaling, RTK Signaling, Fc-epsilon Receptor Signaling Pathway, EGFR Signaling Pathway, Neurotrophin Signaling Pathway, Stem Cell Maintenance, Hepatitis C, Positive Regulation of Response to DNA Damage Stimulus, Interaction of EGFR with phospholipase C-gamma, Thromboxane A2 Receptor Signaling, EGFR Downregulation, S100 Proteins

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents and samples as instructed in the manual. 2. Add 100 µL of sample or positive control to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C. 4. Add 100 µL of prepared primary antibody to each well. 5. Incubate 1 h at RT. 6. Add 100 µL of prepared 1X HRP-Streptavidin to each well. 7. Incubate 1 h at RT. 8. Add 100 µL of TMB One-Step Substrate Reagent to each well.

	9. Incubate 30 min at RT. 10. Add 50 µL of Stop Solution to each well. 11. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Item E, Assay Diluent should be diluted 5-fold with deionized or distilled water before use. 3. Preparation of Positive Control: Briefly spin the Positive Control vial of Item K. Add 500 µL 1x Assay Diluent (Item E, Assay Diluent should be diluted 5-fold with deionized or distilled water before use) into Item K vial to prepare a Positive Control Stock Solution. Dissolve the powder thoroughly by a gentle mix. Add 15 µL prepared Positive Control Stock Solution from the vial of Item K, into a tube with 435 µL 1x Assay Diluent to prepare P-1 (See i. Positive control of part IX.for a typical result). Pipette 300 µL 1x Assay Diluent into each tube. Transfer 150 µL prepared P-1 into a tube with 300 µL 1x Asaay Diluent to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the background. Phospho-EGFR ELISA 6 4. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer. 5. Briefly spin the biotinylated antibody (Item C) before use. Add 100 µL of 1x Assay Diluent into the vial to prepare a biotinylated anti-phosphotyrosine antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days or at -80 °C for one month). The biotinylated phosphotyrosine antibody should be diluted with 1x Assay Diuent and used in step 4 of Part VII Assay Procedure. 6. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 600 fold with 1x Assay Diluent. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 20 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1x Assay Diluent to prepare a 600-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well. P-1 P-2 P-3 P-4 P-5 0 150 µL 15µl Positive Control Stock Solution 435 µL 1x Assay Diluent 150µl 150 µL 150 µL Phospho-EGFR ELISA 7 7. Cell Lysate Buffer should be diluted 2-fold with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors). VII.
Sample Preparation:	Cell lysates - Rinse cells with PBS, making sure to remove any remaining PBS before adding the lysis buffer. Solubilize cells at 4 x 107 cells/mL in 1x Lysis Buffer (we recommend adding protease and phosphatase inhibitors to lysis buffer prior to sample preparation). Pipette up and down to resuspend and incubate the lysates with shaking at 2 - 8° C for 30 minutes.

	Microcentrifuge at 13,000 rpm for 10 minutes at 2 - 8° C, and transfer the supernates into a clean test tube. Lysates should be used immediately or aliquoted and stored at -70 °C. Avoid repeated freeze-thaw cycles. Thawed lysates should be kept on ice prior to use. For the initial experiment, we recommend to do a serial dilution testing such as 5-fold and 100-fold dilution for your cell lysates with Assay Diluent (Item E) before use. Note: The fold dilution of sample used depends on the abundance of phosphorylated proteins and should be determined empiricallys. Phospho-EGFR ELISA 5 More of the sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further. Cell lysate buffer should be diluted 2-fold with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors).
Assay Procedure:	1. Bring all reagents to room temperature (18 - 25 °C) before use. It is recommended that all samples or Positive Control should be run at least in duplicate. 2. Add 100 µL of each sample or positive control into appropriate wells. Cover well with plate holder and incubate for 2.5 hours at room temperature or over night at 4 °C with shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel pipette 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. 4. Add 100 µL of prepared 1X biotinylated anti-phosphotyrosine antibody (Reagent Preparation step 5) to each well. Incubate for 1 hour at room temperature with shaking. 5. Discard the solution. Repeat the wash as in step3. Phospho-EGFR ELISA 8 6. Add 100 µL of prepared 1X HRP-Streptavidin solution (see Reagent Preparation step 6) to each well. Incubate for 45 minutes at room temperature with shaking. 7. Discard the solution. Repeat the wash as in step3. 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	ELISA data analysis: Average the duplicate readings for each sample or positive control then subtract the average blank optical density. i. Positive Control A431 cells were treated with recombinant human EGF at 37 °C for 10 min. Solubilize cells at 4 x 107 cells/mL in lysis buffer. Serial dilutions of lysates were analyzed in this ELISA. Please see step 3 of Part VI Reagent Preparation for detail. 0 0.5 1 1.5 2 2.5 OD =4 50 n m P-1 P-2 P-3 P-4 P-5 Positive control dilution series Assay Diluent Phospho-EGFR ELISA 10 ii. Recombinant Human EGF Stimulation of A431 Cell Lines A431 cells were treated or

Application Details

	untreated with 100 ng/mL recombinant human EGF for 10 min. Cell lysates were analyzed using this phosphoELISA: 0 1 2 3 Untreated EGF treated OD = 4 50 n m Phospho-EGFR ELISA 11 X
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze- thaw cycles.
Storage:	-20 °C
Storage Comment:	Upon receipt, the kit should be stored at -20 °C. Please use within 6 months from the date of shipment. After initial use, Wash Buffer Concentrate (Item B), Assay Diluent (Item E), TMB One-Step Substrate Reagent (Item H), HRP-Streptavidin (Item G), Stop Solution (Item I) and Cell Lysate Buffer (Item J) should be stored at 4 °C to avoid repeated freeze-thaw cycles. Return unused wells to the pouch containing desiccant pack, reseal along entire edge and store at -20 °C. Reconstituted Positive Control (Item K) should be stored at -70 °C.
Expiry Date:	6 months

Images

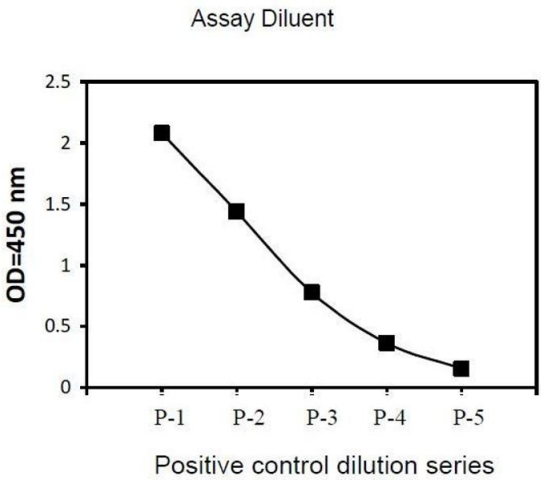

Image 1.

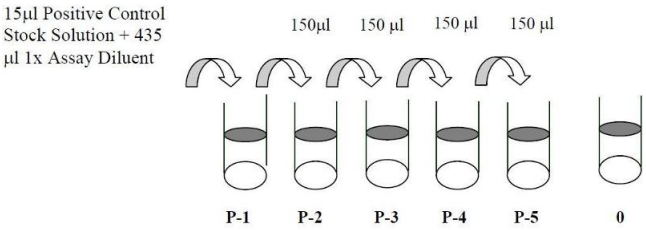

Image 2. This picture shows the preparation of the positive control.

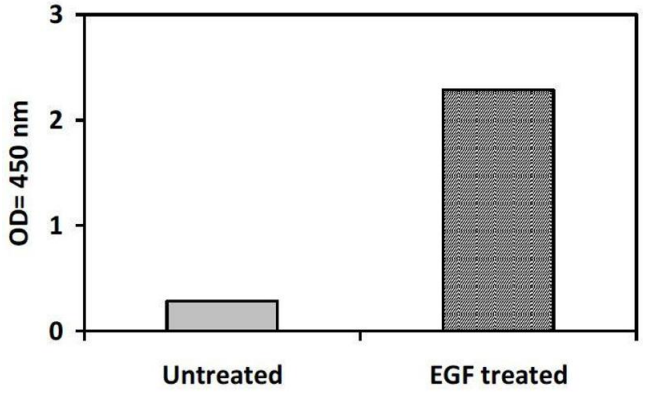

Image 3.