Datasheet for ABIN625225

EGFR ELISA Kit

6 Images

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Overview

Quantity:	96 tests
Target:	EGFR
Binding Specificity:	pTyr992
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Human Phospho-EGFR (Y992) ELISA Kit. This assay semi-quantitatively measures phophorylated EGFR (Tyr992) 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 Phospho-EGFR (pTyr992).
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 • Anti-Phospho Antibody • HRP-Conjugated Secondary Antibody

Product Details

	• Assay Diluent • TMB One-Step Substrate • Stop Solution • Lysis Buffer • Positive Control Sample
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)
Background:	EGFR-Y992
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 (it can be removed by centrifuge if any precipitate in the solution is found). Add 150 µL prepared Positive Control Stock Solution from the vial of Item K, into a tube with 300 µL 1x Assay Diluent to prepare P-1 (See i. Positive control of part IX. TYPICAL DATA for a typical result). Pipette 450 µL 1x Assay Diluent into each tube. Use the Positive Control (1) to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the background. Phospho-EGFR (Tyr 992) ELISA Kit Protocol 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 anti-phospho-EGFR (Tyr 992) (Item C) before use. Add 100 µL of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days. It can be used within one month If store at -80 °C. Avoid repeated freeze-thaw cycles). The detection antibody concentrate should further be diluted 60-folds with 1x Assay Diluent and used in step 4 of Part VII Assay Procedure. 6. Briefly spin the HRP-conjugated anti-rabbit IgG (Item D-1), before use. Pipette up and down to mix gently. HRP- conjugated anti-rabbit IgG concentrate should be diluted 500-folds with 1x Assay Diuent. 7. Cell Lysate Buffer should be diluted 2-folds with deionized or distilled water before use (recommend to add protease and phosphatase inhibitors). 50 µL 150µl Positive Control Stock Solution 300 µL 1x Assay Diluent 50µl 50 µL P-1 P-2 P -3 P-4 P-5 0 50 µL Phospho-EGFR (Tyr 992) ELISA Kit Protocol 7 VII.
Sample Preparation:	Cell lysates - Rinse cells with PBS, making sure to remove any remaining PBS before adding the Cell Lysate Buffer. Solubilize cells at 4 x 107 cells/mL in 1x Cell Lysate Buffer (we recommend adding protease and phosphatase inhibitors to Cell Lysate 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 Phospho-EGFR (Tyr 992) ELISA Kit Protocol 5 abundance of phosphorylated proteins and should be determined empiricallys. 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 anti-phospho-EGFR (Tyr 992) (Reagent Preparation step 5) to each well. Incubate for 1 hour at room temperature with shaking. 5. Discard Discard the solution. Repeat the wash as in step3. 6. Add 100 µL of prepared 500 fold diluted HRP-conjugated anti- rabbit IgG (see Reagent Preparation step 6) to each well. Incubate for over night at 4 °C with shaking. 7. Discard Discard the solution. Repeat the wash as in step3. Phospho-EGFR (Tyr 992) ELISA Kit Protocol 8 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 20 min. Solubilize cells at 4 x 107 cells/mL in Cell Lysate Buffer. Serial dilutions of lysates were analyzed in this ELISA. Please see step 3 of Part VI. Reagent Preparation for detail. Assay Diluent Positive control dilution series O D = 4 5 0 n m 0.1 1 P-1 P-2 P-3 P-4 P-5 Phospho-EGFR

Application Details

	(Tyr 992) ELISA Kit Protocol 10 ii. Recombinant Human EGF Stimulation of A431 Cell Lines A431 cells were treated or untreated with 100 ng/mL recombinant human EGF for 10 min. Cell lysates were analyzed using this phosphoELISA and Western Blot. ELISA Phospho-EGFR (Tyr992) Pan EGFR O D =4 50 n m 0.0 0.5 1.0 1.5 2.0 2.5 Untreated A431 EGF treated A431 Western-Blot hEGF 0 10 0 10 (Min) Anti-phospho-EGFR Anti-EGFR (Tyr992) Phospho-EGFR (Tyr 992) ELISA Kit Protocol 11 iii. SENSITIVITY The A431 cells were treated with 100 ng/mL recombinant human EGF for 20 minutes to induce phosphorylation of EGF R. Serial dilutions of lysates were analyzed in this ELISA and by Western blot. Immunoblots were incubated with anti-phospho-EGFR (Tyr 992). ELISA 100 10 1 0.1 0.01 (µg) O D =4 50 n m 0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 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

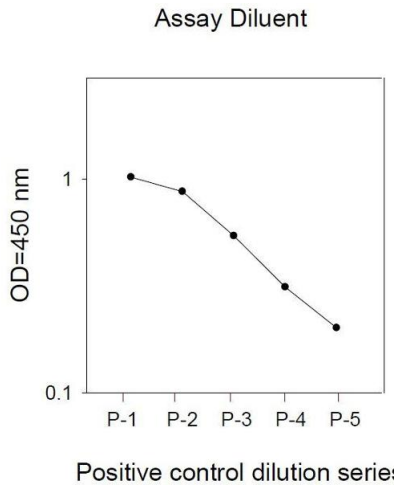

Image 1.

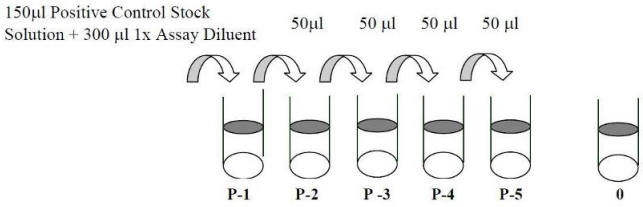

Image 2. This picture shows the preparation of the positive control.

ELISA

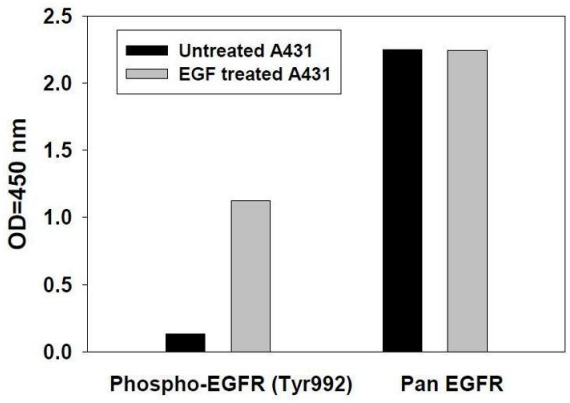

Image 3.

Please check the product details page for more images. Overall 6 images are available for ABIN625225.