Datasheet for ABIN1981821

EGFR ELISA Kit

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Overview

Quantity:	96 tests
Target:	EGFR
Binding Specificity:	phosphorylated
Reactivity:	Human
Method Type:	Cell ELISA
Application:	ELISA

Product Details

Purpose:	Cell-Based Human EGFR (Activated) Phosphorylation ELISA Kit. Suitable for adherent whole cell lines.
Brand:	CellBIND®,RayBio®
Sample Type:	Cell Culture Cells
Analytical Method:	Semi-Quantitative
Detection Method:	Colorimetric
Specificity:	The antibodies provided in this kit recognizes human Tyrosine-phosphorylated-EGFR and total EGFR for comparison.
Characteristics:	• Site and signal pathway-specific • In vitro detection of adherent cell culture • No sample lysis needed • Compatible with a standard ELISA plate reader • Faster results than with ELISA

Product Details

	• Adaptable for high-throughput screening and drug discovery
Components:	• uncoated 96-well Microplate • Wash Buffer A • Wash Buffer B • Fixing Solution • Quenching Buffer • Blocking Buffer • Anti-phospho antibody • Anti-pan antibody • HRP-Conjugated Secondary Antibody • TMB One-Step Substrate • Stop Solution
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:	1956
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:	Uncoated

Application Details

Protocol:

1. Seed 10,000-30,000 cells into each well and incubate overnight.
2. Apply various treatment, inhibitors or activators according to manufacture's instructions.
3. Add 100 µL of Fixing Solution into each well and incubate for 20 min at RT with shaking.
4. Add 200 µL of prepared 1X Quenching Buffer and incubate 20 min at RT.
5. Add 200 µL of Blocking Solution and incubate for 1 h at 37 °C.
6. Add 50 µL of 1X anti-phospho-protein specific antibody or anti-pan-protein specific antibody to each well and incubate for 2 h at RT.
7. Add 50 µL of prepared 1X HRP-Anti-Rabbit or Mouse IgG and incubate for 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:

NOTE: Thaw all reagents to room temperature immediately before use. If wash buffers contain visible crystals, warm to room temperature and mix gently until dissolved.

NOTE: Briefly centrifuge (~1,000g) ITEMS G, H, and I before opening to ensure maximum recovery.

Item, Component, Preparation, Example

A, Uncoated 96 Well Microplate, No Preparation, N/A

B, 20x Wash Buffer A concentrate, Dilute 20-fold with destilled or deionized water, 25 ml of concentrate +475 ml of water = 500 ml of 1x working solution

C, 20x Wash Buffer B concentrate, Dilute 20-fold with destilled or deionized water, 25 ml of concentrate +475 ml of water = 500 ml of 1x working solution

D, Fixing Solution, No Preparation, N/A

E, 30X Quenching Buffer Concentrate, Dilute 30-fold with 1X Wash Buffer A, 1 ml of concentrate + 29 ml of wash buffer = 30 ml of 1X working solution

F, 5X Blocking Buffer Concentrate, Dilute 5-fold with distilled or deionized water, 20 ml of concentrate + 80 ml of water = 100 ml of 1X working solution

Primary Antibodies:

G, 1000x Mouse Anti-phospho (Activated) EGFR Concentrate , Dilute 1000-fold with 1x Blocking Buffer, 2 µL of concentrate + 1998 µL of 1x Blocking Buffer = 2 ml of 1x working solution

H, 1000x Mouse anti-EGFR Concentrate, Dilute 1000-fold with 1x Blocking Buffer, 2 µL of concentrate + 1998 µL of 1x Blocking Buffer = 2 ml of 1x working solution

Secondary Antibody:

I, 1000x HRP Conjugated Anti-Mouse IgG Concentrate, Dilute 1000-fold with 1x Blocking Buffer, 5 µL of concentrate + 4995 µL of 1x Blocking Buffer = 5 ml of 1x working solution

	J, TMB Substrate, No Preparation, N/A K, Stop Solution, No Preparation, N/A
Assay Procedure:	NOTE: ALL incubations and wash steps must be performed under gentle rocking or rotation (~1-2 cycles/sec). 1. Design your experiment. For example, see in Figure 2 below. OPTIONAL: If seeding HUVECs, HMEC-1 or other loosely attached cells, coat the Uncoated 96-Well Microplate (ITEM A) by adding 100 µL poly-L-Lysine (Recommended Sigma Aldrich) into each well and then follow manufacturer's instructions. A pre-coated CellBIND® microplate or other poly-lysine treated tissue culture plate may be used in place of Item A. 2. Seed 100 µL of 30,000 cells into each well of the Uncoated 96-Well Microplate (ITEM A) provided and incubate overnight at 37 °C with 5 % CO2. NOTE: The optimal cell number used will vary on the cell line and the relative amount of protein phosphorylation. More or less cells may be used but this must be determined empirically. NOTE: The cells can be starved ~4-24 hours (depending on cell line) prior to treatment with inhibitors or activators. 3. Apply various treatments, inhibitors (such as siRNA or chemicals) or activators according to manufacturer's instructions and incubate for the desired time points. NOTE: It is recommended to dissolve inhibitors or activators into serum-free cell culture medium before treating the cells (unless otherwise stated in the manufacturer's instructions.) 4. Discard the cell culture medium by flipping the microplate upside down and gently tapping the bottom of the microplate over a sink. 5. Wash by pipetting 200 µL of the prepared 1X Wash Buffer A (ITEM B) into each well. Discard the wash buffer (same as step 4) and wash 2 more times for a total of 3 washes using fresh wash buffer each time. After the final wash, gently blot the microplate onto a paper towel to remove any excess/remaining buffer. NOTE: To avoid cell loss, do not pipette directly onto the cells. Instead, gently dispense the liquid down the wall of cell culture wells. Also avoid the use of vacuum suction or too forcefully tapping the microplate when discarding any solution. 6. Add 100 µL of Fixing Solution (ITEM D) into each well and incubate for 20 minutes at room temperature. NOTE: The fixing solution is used to permeabilize the cells. 7. Repeat wash step 5. 8. Add 200 µL of the prepared 1X Quenching Buffer (ITEM E) into each well and incubate 20 minutes at room temperature. NOTE: The quenching buffer is used to minimize the background response.

Application Details

	9. Wash 4 times with 1X Wash Buffer A. 10. Add 200 µL of the prepared 1X Blocking Buffer (ITEM F) into each well and incubate for 1 hour at 37 °C. 11. Wash 3 times with the prepared 1X Wash Buffer B (ITEM C). NOTE: If needed, the microplate may be stored at -80 °C for several days after this wash. 12. Add 50 µL of the prepared 1X primary antibody (ITEM G or H) into each corresponding well and incubate for 2 hours at room temperature. 13. Wash 4 times with 1X Wash Buffer B. 14. Add 50 µL of 1X HRP Conjugated secondary antibody (ITEM I) into each well and incubate for 1 hour at room temperature. 15. Wash 4 times with 1X Wash Buffer B. 16. Add 100 µL of the TMB Substrate (ITEM J) into each well and incubate for 30 minutes at room temperature in the dark. 17. Add 50 µL of the Stop Solution (ITEM K) into each well. Read at 450 nm immediately.
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 6 months from the date of shipment. Avoid repeated freeze-thaw cycles.
Expiry Date:	6 months

Publications

Product cited in:	Brincks, Kucaba, Legge, Griffith: "Influenza-induced expression of functional tumor necrosis factor-related apoptosis-inducing ligand on human peripheral blood mononuclear cells." in: **Human immunology**, Vol. 69, Issue 10, pp. 634-46, (2008) (PubMed). Janke, Witsch, Mages, Hutloff, Kroczek: "Eminent role of ICOS costimulation for T cells interacting with plasmacytoid dendritic cells." in: **Immunology**, Vol. 118, Issue 3, pp. 353-60, (2006) (PubMed). Coles, Wing, Smith, Coraddu, Greer, Taylor, Weetman, Hale, Chatterjee, Waldmann, Compston: "

Publications

Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis." in: **Lancet (London, England)**, Vol. 354, Issue 9191, pp. 1691-5, (1999) (PubMed).

Caulfield, Fernandez, Sousa, Lane, Lee, Hawrylowicz: "Regulation of major histocompatibility complex class II antigens on human alveolar macrophages by granulocyte-macrophage colony-stimulating factor in the presence of glucocorticoids." in: **Immunology**, Vol. 98, Issue 1, pp. 104-10, (1999) (PubMed).

There are more publications referencing this product on: Product page

Images

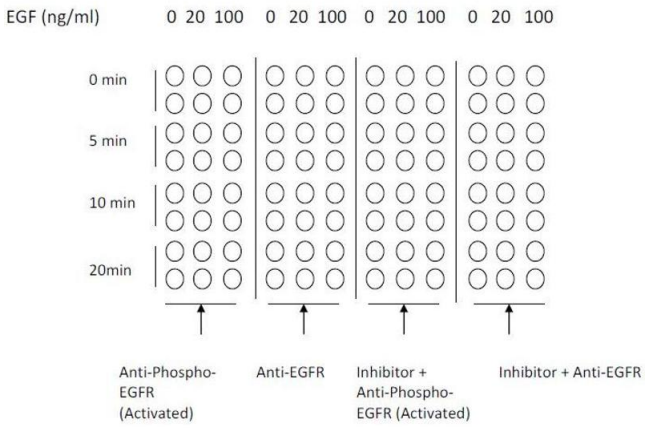


Image 1. Example of how to seed cells for cell-based assay

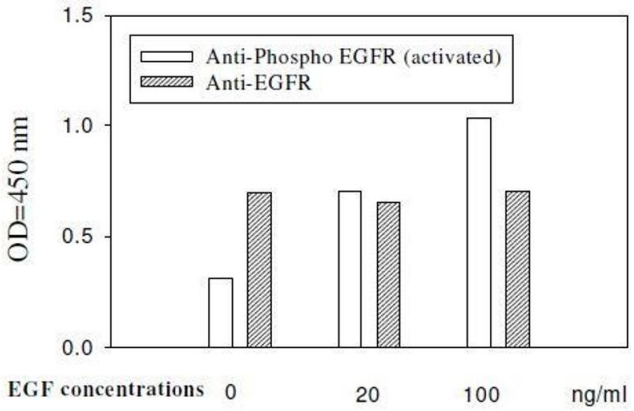
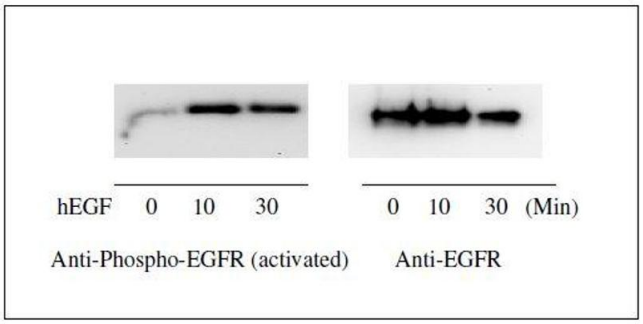


Image 2. A431 cells were stimulated by different concentrations of EGF for 20 min at 37 °C



Western Blotting

Image 3. Western blot analysis of extracts from 100 ng/mL hEGF treated A431 cells. Phospho-EGFR (activated) and EGFR antibodies were used in both detection assays.

Please check the product details page for more images. Overall 4 images are available for ABIN1981821.