Human Phospho-EGFR (Y1173) Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF1095

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human EGFR when phosphorylated at Y1173 in Western blots.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Phosphopeptide containing human EGFR Y1173 site
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

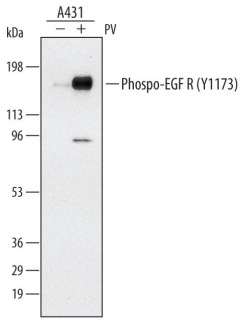
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.2 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	0.5-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
Simple Western	2 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

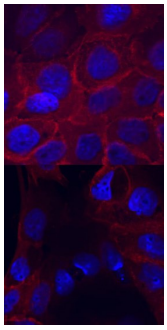
DATA

Western Blot



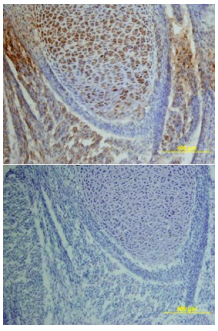
Detection of Human Phospho-EGFR (Y1173) by Western Blot. Western blot shows lysates of A431 human epithelial carcinoma cell line untreated (-) or treated (+) with 100 µM pervanadate (PV) for 10 minutes. PVDF membrane was probed with 0.2 µg/mL of Rabbit Anti-Human Phospho-EGFR (Y1173) Antigen Affinity-purified Polyclonal Antibody, followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # Catalog # HAF008). A specific band was detected for Phospho-EGFR (Y1173) at approximately 185 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



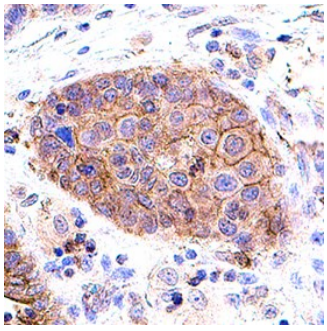
Phospho-EGFR (Y1173) in A431 Human Cell Line. EGFR phosphorylated at Y1173 was detected in immersion fixed A431 human epithelial carcinoma cell line untreated (lower panel) or treated (upper panel) with pervanadate using Rabbit Anti-Human Phospho-EGFR (Y1173) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1095) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # Catalog # NL004) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



Phospho-EGFR (Y1173) in Mouse Embryo. EGFR phosphorylated at Y1173 was detected in immersion fixed frozen sections of mouse embryo using Rabbit Anti-Human Phospho-EGFR (Y1173) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1095) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # Catalog # CTS005) and counterstained with hematoxylin (blue). Lower panel shows a lack of labeling if primary antibodies are omitted and tissue is stained only with secondary antibody followed by incubation with detection reagents. View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

Immunohistochemistry



EGFR in Human Lung Cancer. EGFR was detected in immersion fixed frozen sections of human lung cancer using Rabbit Anti-Human Phospho-EGFR (Y1173) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1095) at 0.5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cell membrane in cancer cells. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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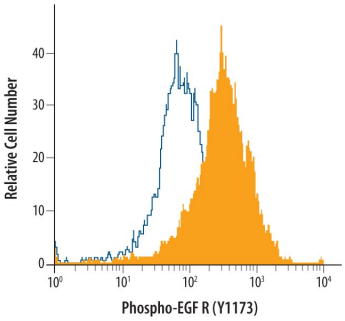
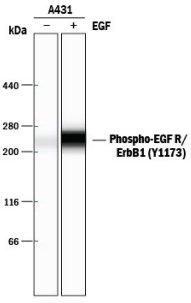
Intracellular Staining by Flow Cytometry



Detection of Phospho-EGFR (Y1173) in A431 Human Cell Line by Flow Cytometry. A431 human epithelial carcinoma cells were untreated (open histogram), or treated for 5 minutes with 100 ng/mL Recombinant Human EGF (Catalog # Catalog # 236-EG; filled histogram) then stained with Rabbit Anti-Human Phospho-EGFR (Y1173) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1095), followed by Phycoerythrin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # Catalog # F0110). To facilitate intracellular staining, cells were fixed with para-formaldehyde and permeabilized with saponin.

Simple Western



Detection of Human Phospho-EGFR (Y1173) by Simple Western™. Simple Western lane view shows lysates of A431 human epithelial carcinoma cell line untreated (-) or treated (+) with 10 ng/mL Recombinant Human EGF (Catalog # Catalog # 236-EG) for 5 minutes, loaded at 0.2 mg/mL. A specific band was detected for Phospho-EGFR (Y1173) at approximately 265 kDa (as indicated) using 2 µg/mL of Rabbit Anti-Human Phospho-EGFR (Y1173) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1095). This experiment was conducted under reducing conditions and using the 66-440 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Epidermal growth factor receptor (EGFR, also known as ErbB1 and HER1) is the founding member of the ErbB family of receptor tyrosine kinases. Ligand binding induces receptor dimerization and autophosphorylation on multiple tyrosine residues. Phosphorylation of Y1173 creates a binding site for the protein tyrosine phosphatase SHP-1, leading to attenuation of receptor signaling.

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