

Phospho-EGFR (Tyr1068) Monoclonal Antibody (EP38Y)

Catalog NumberMA5-14485

Product data sheet

Details		Species Reactivity	
Size	1 mL	Species reactivity	Human, Mouse
Host/Isotope	Rabbit / IgG	Published species	Human, Not Applicable
Class	Monoclonal	Tested Applications	
Type	Antibody	Immunohistochemistry (Paraffin) (IHC (P))	1:100
Clone	EP38Y	Western Blot (WB)	1:250
Immunogen	A synthetic phospho-peptide corresponding to residues surrounding Tyr1068 of human EGFR	Immunocytochemistry (ICC/IF)	1:250
		Published Applications	
Conjugate	Unconjugated	Immunohistochemistry (Paraffin) (IHC (P))	See 2 publications below
Form	Liquid	Immunohistochemistry (IHC)	See 1 publications below
Concentration	Conc. Not Determined	* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.	
Storage Conditions	4° C		

Product specific information

MA5-14485 targets Epidermal Growth Factor Receptor in IHC (P) applications and shows reactivity with Human samples. The MA5-14485 immunogen is a synthetic phospho-peptide corresponding to residues surrounding Tyr1068 of human EGFR.

Background/Target Information

EGFR (Epidermal growth factor receptor, HER1, ErbB1) is encoded by the EGFR gene located on chromosome 7 in humans. EGFR belongs to the HER /ERBB family of proteins that includes three other receptor tyrosine kinases, ERBB2, ERBB3, ERBB4. EGFR is a transmembrane receptor and binding of its cognate ligands such as EGF (Epidermal Growth Factor) and TGF alpha (Transforming Growth Factor alpha) to the extracellular domain leads to EGFR dimerization followed by autophosphorylation of the tyrosine residues in the cytoplasmic domain. Phosphorylation of EGFR at certain residues is also mediated by Src-non-receptor kinase. EGFR activation signals multiple downstream signaling cascades such as the Ras - ERK, PI3-K - Akt, Jak - STAT and PKC pathways that help in growth and proliferation of cells. Phosphorylation of EGFR at Y1086 specifically allows binding of the adaptor protein GRB2, leading to activation of the MAPK pathway. Upon receptor activation and signaling, EGFR is endocytosed and targeted for degradation or recycling. Mutations in the EGFR gene are associated with lung cancer and multiple alternatively spliced transcript variants encode different protein isoforms of EGFR have been found. Increased production or activation of EGFR has been associated with poor prognosis in a variety of tumors. Moreover, EGFR overexpression is observed in tumors of the head and neck, brain, bladder, stomach, breast, lung, endometrium, cervix, vulva, ovary, esophagus, stomach and in squamous cell carcinoma.

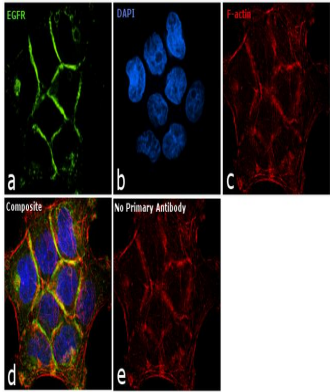
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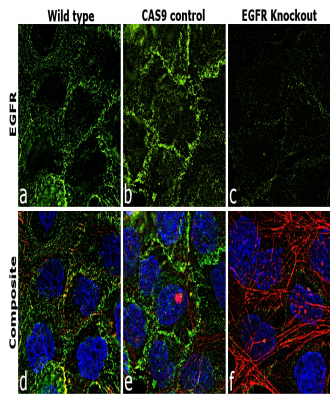
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Product Images For Phospho-EGFR (Tyr1068) Monoclonal Antibody (EP38Y)



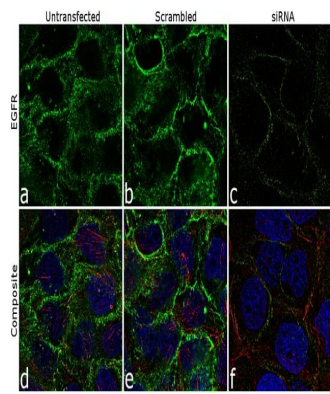
Phospho-EGFR (Tyr1068) Antibody (MA5-14485) in ICC/IF

Immunofluorescence analysis of EGFR was performed using 90% confluent log phase A431 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with Phospho-EGFR (Tyr1068) Rabbit Monoclonal antibody (Product # MA5-14485) at 1:250 dilution in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034) at a dilution of 1:2,000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing membrane localization. Panel e shows the no primary antibody control. The images were captured at 60X magnification.



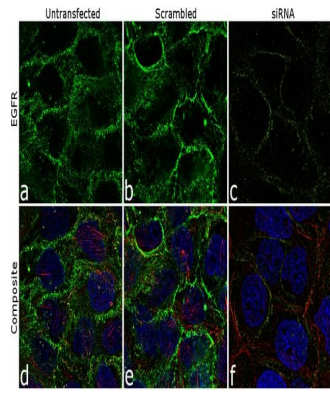
Phospho-EGFR (Tyr1068) Antibody (MA5-14485)

Altered expression of target protein upon Knockout demonstrates antibody specificity. Immunofluorescence analysis of EGFR using Phospho-EGFR (Tyr1068) Rabbit monoclonal Antibody (Product # MA5-14485) shows no expression in A-431 EGFR knockout cells. {KO}



Phospho-EGFR (Tyr1068) Antibody (MA5-14485) in ICC/IF

Knockdown of EGFR was achieved by transfecting A431 cells with EGFR specific siRNA (Silencer® select Product # s563, s564 and s565). Immunofluorescence analysis was performed on A431 cells (untransfected, panel a,d), transfected with non-specific scrambled siRNA (panels b,e) and transfected with EGFR specific siRNA (panel c,f). Cells were fixed, permeabilized, and labelled with Phospho-EGFR (Tyr1068) Rabbit Monoclonal Antibody (Product # MA5-14485, 5 µg/mL), followed by Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034, 1:2,000). Nuclei (blue) were stained using SlowFade® Gold Antifade Mountant with DAPI (Product # S36938), and Rhodamine Phalloidin (Product # R415, 1:300) was used for cytoskeletal F-actin (red) staining. Loss of signal was observed upon siRNA mediated knockdown (panel c,f) confirming specificity of the antibody to EGFR (green). The images were captured at 60X magnification.



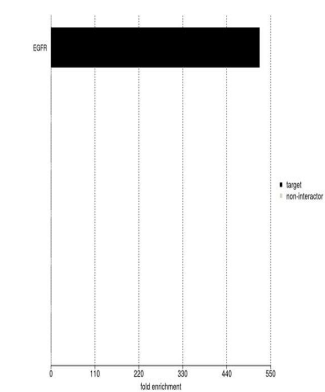
Phospho-EGFR (Tyr1068) Antibody (MA5-14485)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. A-431 cells were transfected with EGFR siRNA and decrease of signal was observed in immunofluorescence application using Phospho-EGFR (Tyr1068) Rabbit Monoclonal antibody (Product # MA5-14485). {KD}

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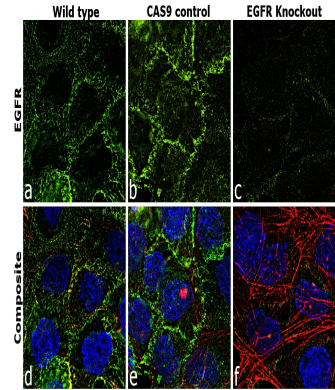


Phospho-EGFR (Tyr1068) Antibody (MA5-14485)

IP-MS enrichment of EGFR (LFQ intensity): EGFR was enriched 522-fold from A549 lysate compared to background proteins, using the optimized IP-MS workflow with Pierce MS-Compatible Magnetic IP Kit protein A/G (Product # 90409) and Phospho-EGFR (Tyr1068) antibody (Product # MA5-14485). See more information on IP-MS verification of antibody selectivity. {IP-MS}

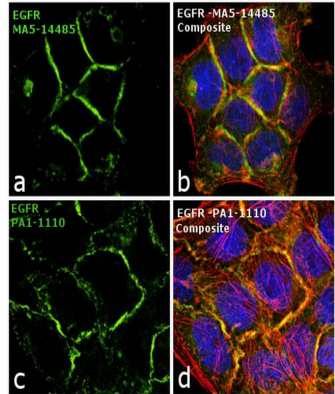
Phospho-EGFR (Tyr1068) Antibody (MA5-14485) in ICC/IF

Immunofluorescence analysis of EGFR was performed using 70% confluent log phase A-431 cells (Wild type, panels a,d), CAS9 control (panels b,e) and EGFR Knockout (panels c,f). The cells were fixed, permeabilized, and labelled with Phospho-EGFR (Tyr1068) Rabbit Monoclonal Antibody(Product # MA5-14485, 1:100), followed by Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034, 1:2,000). Nuclei (blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938) and Rhodamine Phalloidin (Product # R415, 1:300) was used for cytoskeletal F-actin (red) staining. Loss of signal was observed in EGFR Knockout cells (panel c,f) confirming specificity of the antibody to EGFR(green). The images were captured at 60X magnification.



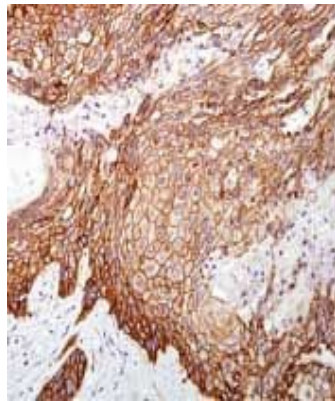
Phospho-EGFR (Tyr1068) Antibody (MA5-14485)

Antibody specificity was demonstrated by showing that antibodies raised against the same target protein perform similarly. Immunofluorescence of EGFR using Phospho-EGFR (Tyr1068) Antibody, Rabbit Monoclonal (Product # MA5-14485) along with another EGFR antibody (Product # PA1-1110) shows similar pattern of EGFR expression in A-431 cells. {IAV}



Phospho-EGFR (Tyr1068) Antibody (MA5-14485) in IHC (P)

Formalin-fixed, paraffin-embedded human squamous cell carcinoma stained with rabbit monoclonal Phospho-EGFR (Tyr1068) antibody using peroxidase-conjugate and DAB chromogen. Note membrane staining of tumor cells.



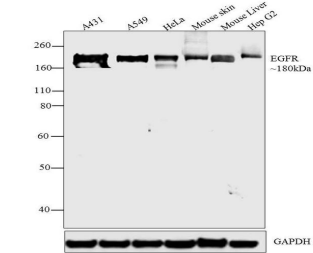
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Phospho-EGFR (Tyr1068) Antibody (MA5-14485) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of A431 (Lane 1), A549 (Lane 2), HeLa (lane 3), Mouse Skin (Lane 4), Mouse Liver (Lane 5) and Hep G2 (Lane 6). The blots were probed with Phospho-EGFR (Tyr1068) Rabbit Monoclonal Antibody (Product # MA5-14485, 1:250 dilution) and detected by chemiluminescence Goat anti-Rabbit IgG (H+L) Secondary Antibody, HRP conjugate (Product # G-21234, 1:5,000 dilution). A 180 kDa band corresponding to EGFR was observed across cell lines and tissues tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 10 % Bis-Tris gel (Product # NP0301BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with by overnight wet transfer method. The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



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PubMed References For Phospho-EGFR (Tyr1068) Monoclonal Antibody (EP38Y)

2 Immunohistochemistry (Paraffin) References

Species / Dilution	Summary
Human / 1:30	MA514485 was used in immunohistochemistry - paraffin section to characterize and present the first case of fibroblastic reticular cell tumor of the breast
	Experimental and therapeutic medicine (2016; 11: 561) "Fibroblastic reticular cell tumor of the breast: A case report and review of the literature." Author(s):Li H,Shen P,Liang Y,Zhang F PubMed Article URL: http://dx.doi.org/10.3892/etm.2015.2922
Not Applicable / 1:50	MA5-14485 was used in immunohistochemistry - paraffin section to analyze triple negative breast carcinomas and their correlation with mesothelin expression and basal-like phenotype, decreased survival, and distant metastases
	PloS one (2015; 9:) "Mesothelin expression in triple negative breast carcinomas correlates significantly with basal-like phenotype, distant metastases and decreased survival." Author(s):Tozbikian G,Brogi E,Kadota K,Catalano J,Akram M,Patil S,Ho AY,Reis-Filho JS,Weigelt B,Norton L,Adusumilli PS,Wen HY PubMed Article URL: http://dx.doi.org/10.1371/journal.pone.0114900

1 Immunohistochemistry References

Species / Dilution	Summary
Human / 1:100	MA5-14485 was used in immunohistochemistry to report on a case of sporadic haemangioblastoma of the kidney with rhabdoid features and focal CD10 expression
	Diagnostic pathology (2012; 7:) "Sporadic haemangioblastoma of the kidney with rhabdoid features and focal CD10 expression: report of a case and literature review." Author(s):Yin WH,Li J,Chan JK PubMed Article URL: http://dx.doi.org/10.1186/1746-1596-7-39

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