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Product data sheet

EGFR Monoclonal Antibody (H11)

Catalog Number MA5-13070

Details		Species Reactivity	
Size	500 μL	Species reactivity	Human, Mouse
Host/Isotope	Mouse / IgG1, kappa	Published species	Human, Mouse, Not Applicable
Class	Monoclonal	Tested Applications	Dilution *
Туре	Antibody	Flow Cytometry (Flow)	0.5-1 µg/test
Clone	H11	Immunohistochemistry (Paraffin) (IHC (P))	2-4 µg/mL
Immunogen	HC2 20 d2 cells	Immunoprecipitation (IP)	2 µg/mL
Conjugate	Unconjugated	Western Blot (WB)	0.5-1.0 μg/mL
Form	Liquid	Immunocytochemistry (ICC/IF)	2 µg/mL
Concentration	0.2 mg/mL	Published Applications	
Purification	Protein G	Immunohistochemistry (IHC)	See 2 publications below
Storage buffer	PBS, pH 7.4, with 0.2% BSA	Western Blot (WB)	See 29 publications below
Contains	0.09% sodium azide	Miscellaneous PubMed (Misc)	See 1 publications below
Storage Conditions	4° C	Immunohistochemistry (Paraffin) (IHC (P))	See 1 publications below
		Immunocytochemistry (ICC/IF)	See 5 publications below
		In vitro Assay (IV)	See 1 publications below
		Neutralization (Neu)	See 2 publications below
		Immunoprecipitation (IP)	See 3 publications below
		ELISA (ELISA)	See 2 publications below
		Flow Cytometry (Flow)	See 3 publications below

* 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.

See 1 publications below

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Product specific information

MA5-13070 targets Epidermal Growth Factor Receptor in FACS, ICC/IF, IHC (P), IP, and WB applications and shows reactivity with Human and Mouse samples. This antibody is not recommended for mouse lymph node tissue or human breast carcinoma in IHC applications. The MA5-13070 immunogen is hC2 20 d2 cells.

Gel Shift (GS)

Background/Target Information

EGFR, epidermal growth factor receptor, is a receptor tyrosine kinases that signals in response to various growth factors. Overexpression has been linked to numerous types of cancer and EGFR is the target of both biological and small molecular therapeutics. EGFR 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. 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.

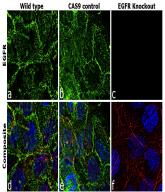
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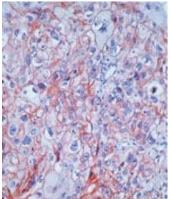
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Product Images For EGFR Monoclonal Antibody (H11)



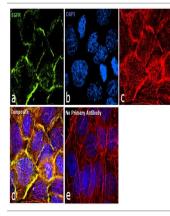
EGFR Antibody (MA5-13070)

Altered expression of target protein upon Knockout demonstrates antibody specificity. Immunofluorescence analysis of EGFR using Anti-EGFR Mouse monoclonal Antibody (Product # MA5-13070) shows no expression in A-431 EGFR knockout cells. {KO}



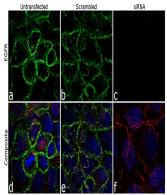
EGFR Antibody (MA5-13070) in IHC (P)

Formalin-fixed, paraffin-embedded human squamous cell carcinoma of lung stained with EGFR antibody using peroxidase-conjugate and AEC chromogen. Note cell membrane staining of tumor cells.



EGFR Antibody (MA5-13070) in ICC/IF

Immunofluorescence analysis of EGFR was performed using 90% confluent log phase A-431 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 EGFR Mouse monoclonal antibody (Product # MA5-13070) at 2 µg/mL in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Mouse IgG (H+L) Superclonal[™] Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:2000 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.



EGFR Antibody (MA5-13070)

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 EGFR Mouse Monoclonal antibody (Product # MA5-13070). {KD}

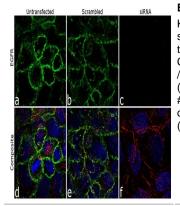
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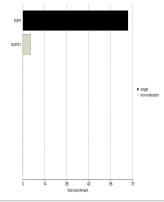
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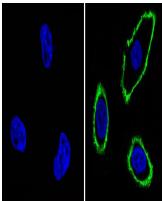
EGFR Antibody (MA5-13070) in ICC/IF

Knockdown of EGFR was achieved by transfecting A-431 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 EGFR Mouse monoclonal Antibody (Product # MA5-13070, 5 µg /mL), followed by Goat anti-Mouse IgG (H+L) Superclonal[™] Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175, 1:2000). 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.



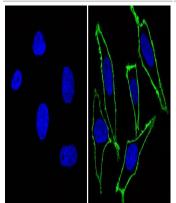
EGFR Antibody (MA5-13070)

IP-MS enrichment of EGFR (LFQ intensity): EGFR was enriched 67-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 EGFR antibody (Product # MA5-13070). The STRING database (www.string-db.org) was used to identify the protein interactor list. See more information on IP-MS verification of antibody selectivity. {IP-MS}



EGFR Antibody (MA5-13070) in ICC/IF

Immunofluorescent analysis of Epidermal Growth Factor Receptor (green) showing staining in the membrane of A431 cells (right) compared to a negative control without primary antibody (left). Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with an Epidermal Growth Factor Receptor monoclonal antibody (Product # MA5-13070) in 3% BSA-PBS at a dilution of 1:100 and incubated overnight at 4 °C in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody in PBS at room temperature in the dark. F-actin (red) was stained with a fluorescent red phalloidin and nuclei (blue) were stained with Hoechst or DAPI. Images were taken at a magnification of 60x.



EGFR Antibody (MA5-13070) in ICC/IF

Immunofluorescent analysis of Epidermal Growth Factor Receptor (green) showing staining in the membrane of Hela cells (right) compared to a negative control without primary antibody (left). Formalin-fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 5-10 minutes and blocked with 3% BSA-PBS for 30 minutes at room temperature. Cells were probed with an Epidermal Growth Factor Receptor monoclonal antibody (Product # MA5-13070) in 3% BSA-PBS at a dilution of 1:100 and incubated overnight at 4 °C in a humidified chamber. Cells were washed with PBST and incubated with a DyLight-conjugated secondary antibody in PBS at room temperature in the dark. F-actin (red) was stained with a fluorescent red phalloidin and nuclei (blue) were stained with Hoechst or DAPI. Images were taken at a magnification of 60x.

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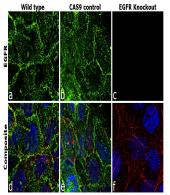
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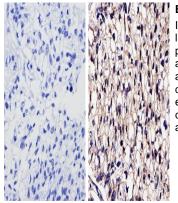
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EGFR Antibody (MA5-13070) in ICC/IF

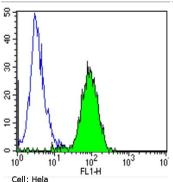


Immunofluorescence analysis of EGFR was performed using 70% confluent log phase A-431 cells (WIId type, panels a,d), CAS9 control (panels b,e) and EGFR Knockout (panels c,f). The cells were fixed, permeabilized, and labelled with EGFR Mouse Monoclonal Antibody(Product # MA5-13070, 5 µg/mL), followed by Goat anti-Mouse IgG (H+L) Superclonal[™] Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175, 1:2000). 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.

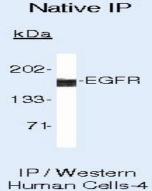


EGFR Antibody (MA5-13070) in IHC (P)

Immunohistochemistry analysis of EGFR showing staining in the membrane and cytoplasm of paraffin-treated human lung carcinoma (right) compared with a negative control in the absence of primary antibody (left). To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with a Epidermal Growth Factor Receptor monoclonal antibody (Product # MA5-13070) diluted by 3% BSA-PBS at a dilution of 1:50 overnight at 4°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.



Concentration: 0.5µg/test (100µl) Theory location : Membrane/Cytoplasm



EGFR Antibody (MA5-13070) in Flow

Flow cytometry analysis of Epidermal Growth Factor Receptor in Hela cells compared to an isotype control (blue). Cells were harvested, adjusted to a concentration of 1-5x10^6 cells/mL, fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with a Epidermal Growth Factor Receptor monoclonal antibody (Product # MA5-13070) at a dilution of 0.5 µg/test for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.

EGFR Antibody (MA5-13070) in IP

Immunoprecipitation of Epidermal Growth Factor Receptor using Epidermal Growth Factor Receptor Monoclonal Antibody (Product # MA5-13070) on Native Human A431 Cells.

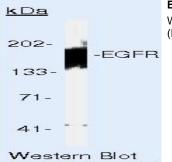
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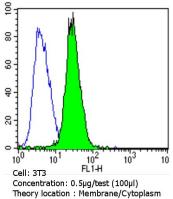
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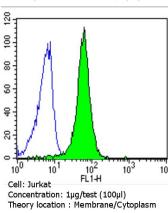


EGFR Antibody (MA5-13070) in WB

Western blot of Epidermal Growth Factor Receptor using Epidermal Growth Factor Receptor Monoclonal Antibody (Product # MA5-13070) on Human Epidermoid.

EGFR Antibody (MA5-13070) in Flow

Flow cytometry analysis of Epidermal Growth Factor Receptor in NIH/3T3 cells compared to an isotype control (blue). Cells were harvested, adjusted to a concentration of 1-5x10^6 cells/mL, fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with a Epidermal Growth Factor Receptor monoclonal antibody (Product # MA5-13070) at a dilution of 0.5 µg/test for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.



EGFR Antibody (MA5-13070) in Flow

Flow cytometry analysis of Epidermal Growth Factor Receptor in Jurkat cells compared to an isotype control (blue). Cells were harvested, adjusted to a concentration of 1-5x10^6 cells/mL, fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with a Epidermal Growth Factor Receptor monoclonal antibody (Product # MA5-13070) at a dilution of 1 µg/test for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.

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2 Immunohistochemist	For EGFR Monoclonal Antibody (H11) rv References
Species / Dilution	Summary
	MA5-13070 was used in immunohistochemistry to describe an EGF receptor mutant with tandem kinase domain duplication in glioblastoma multiforme biopsies and cell lines
Mouse / 1:500	Oncogene (2010; 29: 855) "Activity and cellular localization of an oncogenic glioblastoma multiforme-associated EGF receptor mutant possessing a duplicated kinase domain." Author(s):Ozer BH,Wiepz GJ,Bertics PJ PubMed Article URL:http://dx.doi.org/10.1038/onc.2009.385
	MA5-13070 was used in Immunohistochemistry-immunofluorescence to indicate that the combination of IMP3 and BCL-2 may be of diagnostic utility in distinguishing between ISK and SCCIS in daily clinical practice.
Human / 1:50	Journal of cutaneous pathology (2018; 45: 603) "Distinguishing between irritated seborrheic keratosis and squamous cell carcinoma in situ using BCL-2 and IMP3 immunohistochemistry." Author(s):Richey JD,Deng AC,Dresser K,O'Donnell P,Cornejo KM PubMed Article URL:http://dx.doi.org/10.1111/cup.13269
29 Western Blot Refere	ences
Species / Dilution	Summary
	MA5-13070 was used in western blot to study the effect of miR-200 expression on the epithelial-to-mesenchymal transition and resistance to EGFR therapy in bladder cancer cells
Human / Not Cited	Clinical cancer research : an official journal of the American Association for Cancer Research (2009; 15: 5060) "miR-200 expression regulates epithelial-to-mesenchymal transition in bladder cancer cells and reverses resistance to epidermal growth factor receptor therapy." Author(s):Adam L,Zhong M,Choi W,Qi W,Nicoloso M,Arora A,Calin G,Wang H,Siefker-Radtke A,McConkey D,Bar-Eli M, Dinney C PubMed Article URL:http://dx.doi.org/10.1158/1078-0432.CCR-08-2245
Human / Not Cited	MA513070 was used in immunohistochemistry and western blot to determine that MUC1 stimulates epidermal growth factor receptor expression and function in endometrial cancer
	Oncotarget (2016; 7: 32796) "MUC1 stimulates EGFR expression and function in endometrial cancer." Author(s):Engel BJ,Bowser JL,Broaddus RR,Carson DD PubMed Article URL:http://dx.doi.org/10.18632/oncotarget.8743
	MA5-13070 was used in western blot to study the role of cortactin overexpression in increasing invasion potential in oral squamous cell carcinoma
Human / Not Cited	Pathology oncology research : POR (2010; 16: 523) "Overexpression of cortactin increases invasion potential in oral squamous cell carcinoma." Author(s):Yamada S,Yanamoto S,Kawasaki G,Mizuno A,Nemoto TK PubMed Article URL:http://dx.doi.org/10.1007/s12253-009-9245-y
	MA5-13070 was used in western blot to study whether gene silencing by vector-mediated RNAi inhibition of EGFR expression can reduce the growth of cultured human glioma cells
Human / 1:50	Molecular therapy : the journal of the American Society of Gene Therapy (2005; 12: 803) "Herpes simplex virus 1 amplicon vector-mediated siRNA targeting epidermal growth factor receptor inhibits growth of human glioma cells in vivo." Author(s):Saydam O,Glauser DL,Heid I,Turkeri G,Hilbe M,Jacobs AH,Ackermann M,Fraefel C PubMed Article URL:http://dx.doi.org/10.1016/j.ymthe.2005.07.534
Human / 1:3000	MA5-13070 was used in western blot to study the mechanism of resistance to the EGFR tyrosine kinase inhibitor Gefitinit in bladder cancer cells
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