





ERK1/ERK2 Monoclonal Antibody (3F8B3)

Catalog Number MA5-15605 Product data sheet

Details	
Size	100 μg
Host/Isotope	Mouse / IgG2b
Class	Monoclonal
Туре	Antibody
Clone	3F8B3
Immunogen	Purified recombinant fragment of human MAPK expressed in E. Coli.
Conjugate	Unconjugated
Form	Liquid
Concentration	1 mg/mL
Purification	Affinity chromatography
Storage buffer	PBS with 1mg/mL BSA
Contains	0.05% sodium azide
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.

Species Reactivity	
Species reactivity	Human, Mouse, Rat
Published species	Human, Not Applicable
Tested Applications	Dilution *
Immunohistochemistry (IHC)	1:200-1:1,000
Western Blot (WB)	1:1,000
Immunocytochemistry (ICC/IF)	1:100-1:300

Published Applications	
Western Blot (WB)	See 1 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.

Product specific information

MA5-15605 targets p44/42 MAPK (Erk1/2) in FACS, IHC, WB and IF applications and shows reactivity with Human and mouse samples.

Background/Target Information

ERK1 and ERK2 are widely expressed and are involved in the regulation of meiosis, mitosis, and postmitotic functions in differentiated cells. Many different stimuli, including growth factors, cytokines, virus infection, ligands for heterotrimeric guanine nucleotide-binding protein (G protein)-coupled receptors and transforming agents, activate the ERK1 and ERK2 pathways. When growth factors bind to the receptor tyrosine kinase, Ras interacts with Raf, the serine/threonine protein kinase and activates it as well. Once actived, Raf phosphorylates serine residue in 2 further kinases, MEK1/2, which in turn phosphorylates tyrosine/threonine in extracellular-signal regulated kinase (ERK) 1/2. Upon activation, the ERKs either phosphorylate a number of cytoplasmic targets or migrate to the nucleus, where they phosphorylate and activate a number of transcription factors such as c-Fos and Elk-1.

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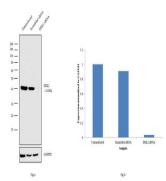
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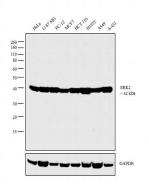


Product Images For ERK1/ERK2 Monoclonal Antibody (3F8B3)



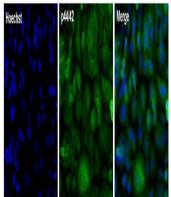
ERK1/ERK2 Antibody (MA5-15605)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. A549 cells were transfected with alpha ERK1/ERK2 siRNA and loss of signal was observed in Western Blot using Anti-ERK1/ERK2 Monoclonal Antibody (Product # MA5-15605). {KD}



ERK1/ERK2 Antibody (MA5-15605) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of HeLa (Lane 1), U-87 MG (Lane 2), PC-12 (Lane 3), MCF7 (Lane 4), HCT 116 (Lane 5), H1975 (Lane 6), A549 (Lane 7) and A-431 (Lane 8). The blot was probed with Anti-ERK1/ERK2 Monoclonal Antibody (Product # MA5-15605, 1:1000 dilution) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg/mL, 1:4000 dilution). A 42 kDa band corresponding to ERK2 was observed across the cell lines tested.



ERK1/ERK2 Antibody (MA5-15605) in ICC/IF

Immunofluorescent analysis of p44, 42 MAPK (Erk1/2) in HeLa cells. The cells were fixed with 4% paraformaldehyde in PBS for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 for 15 minutes, and blocked with 3% BSA for 30 minutes at room temperature. Cells were stained with a p44/42 MAPK (Erk1/2) mouse monoclonal antibody (Product # MA5-15605) at a dilution of 1:250 in blocking buffer for 1 hour at room temperature, and then incubated with a Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:1000 for at least 30 minutes at a room temperature in the dark (green). Nuclei (blue) were stained with Hoechst 33342 (Product # 62249). Images were taken on a Thermo Scientific ToxInsight Instrument at 20X magnification.



ERK1/ERK2 Antibody (MA5-15605) in IHC (P)

Immunohistochemistry was performed on human liver tissue. Tissue was deparaffinized with xylene, followed by rehydration in sequential washes of 100% ethanol, 95% ethanol, 80% ethanol, and water. To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0) and heated for 20 min. Following antigen retrieval, tissues were blocked in a 10% goat serum (Product # 31872) in wash buffer solution for 30-60 minutes at room temperature. Tissue was then probed with a p42, 44 ERK (1, 2) Mouse Monoclonal antibody (Product # MA5-15605) at a dilution of 1:100 in 10% goat serum in wash buffer overnight at 4°C in a humidified chamber. Negative control tissue received no primary antibody. Tissues were washed extensively with PBST, and detection was performed using a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:500 followed by colorimetric detection using metal enhanced DAB. Tissues were then counterstained with hematoxylin and prepped for mounting and imaging.

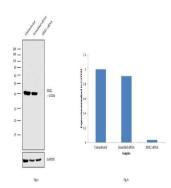
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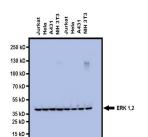
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ERK1/ERK2 Antibody (MA5-15605) in WB

Knockdown of ERK1/ERK2 was achieved by transfecting A549 cells with ERK1/ERK2 specific validated siRNAs (Silencer® select Product # s11140, s11138). Western blot analysis (Fig. a) was performed using whole cell extracts from the ERK1/ERK2 knockdown cells (lane 3), non-specific scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blot was probed with ERK1/ERK2 Monoclonal Antibody (Product # MA5-15605, 1: 1000 dilution) and Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg/mL, 1:4000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to GAPDH.



ERK1/ERK2 Antibody (MA5-15605) in WB

Western blot analysis of p42, 44 ERK (1, 2) was performed by loading 20 µg of the indicated whole cell lysates and 5 µl of PageRuler Plus Prestained Protein Ladder (Product # 26619) per well onto a 4-20% Tris-Glycine polyacrylamide gel (Product # WT4202BX10). Proteins were transferred to a nitrocellulose membrane using the G2 Blotter (Product # 62288), and blocked with 5% Milk in TBST for 1 hour at room temperature. p42, 44 ERK (1, 2) was detected at 42kDa using a p42, 44 ERK (1, 2) mouse monoclonal antibody (Product # MA5-15605) at a dilution of 1:1000 in blocking buffer for 1 hour at room temperature on a rocking platform, followed by a Goat anti-Mouse IgG (H+L) SuperclonalTM Secondary Antibody, HRP conjugate (Product # A28177) at a dilution of 1:1000 for at least 30 minutes at room temperature. Chemiluminescent detection was performed using SuperSignal West Pico (Product # 34078).

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PubMed References	For ERK1/ERK2 Monoclonal Antibody (3F8B3)
1 Western Blot Referen	ces
Species / Dilution	Summary
Human / 1:1,000 Th "T hy Au	MA5-15605 was used in Western Blotting to reveal prominent underlying mechanisms of the AAE6's enhanced oncogenic potential.
	The Journal of general virology (2017; 98: 2310) "Two common variants of human papillomavirus type 16 E6 differentially deregulate sugar metabolism and hypoxia signalling in permissive human keratinocytes." Author(s):Cuninghame S,Jackson R,Lees SJ,Zehbe I PubMed Article URL:http://dx.doi.org/10.1099/jgv.0.000905

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