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RAP1A Monoclonal Antibody (1D2-1C64)

Catalog Number **MA1-013**

Product data sheet

Details	
Size	100 µg
Host/Isotope	Mouse / IgG2a
Class	Monoclonal
Type	Antibody
Clone	1D2-1C64
Immunogen	Recombinant full-length human Rap1
Conjugate	Unconjugated
Form	Liquid
Concentration	1 mg/mL
Purification	Protein A
Storage buffer	PBS with 30% glycerol, 1mg/mL BSA
Contains	0.05% sodium azide
Storage Conditions	-20°C

Species Reactivity	
Species reactivity	Human, Mouse
Published species	Human

Tested Applications	Dilution *
Immunohistochemistry (Paraffin) (IHC (P))	1:100-1:1,000
Immunoprecipitation (IP)	2 µg
Western Blot (WB)	1:500-1:1,000
Immunocytochemistry (ICC/IF)	1:10-1:200

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

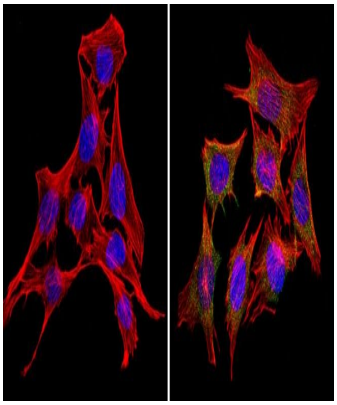
Western blot analysis of MA1-013 detects an ~21kDa protein.

Background/Target Information

The product of this gene belongs to the family of RAS-related proteins. These proteins share approximately 50% amino acid identity with the classical RAS proteins and have numerous structural features in common. The most striking difference between RAP proteins and RAS proteins resides in their 61st amino acid: glutamine in RAS is replaced by threonine in RAP proteins. The product of this gene counteracts the mitogenic function of RAS because it can interact with RAS GAPs and RAF in a competitive manner. Two transcript variants encoding the same protein have been identified for this gene.

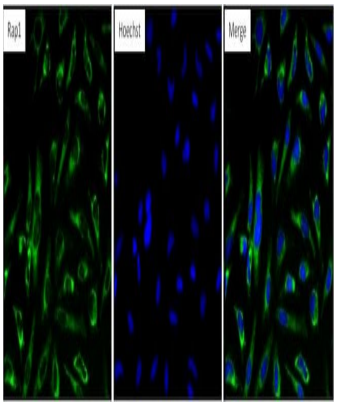
For Research Use Only. Not for use in diagnostic procedures. Not for resale without express authorization.

Product Images For RAP1A Monoclonal Antibody (1D2-1C64)



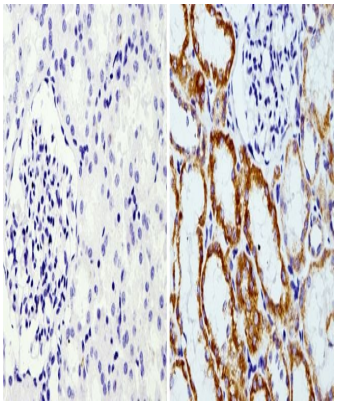
RAP1A Antibody (MA1-013) in ICC/IF

Immunofluorescent analysis of Rap1 (green) showing staining in the in the cytoplasm of C2C12 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 a Rap1 monoclonal antibody (Product # MA1-013) in 3% BSA-PBS at a dilution of 1:20 and incubated overnight at 4ºC 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.



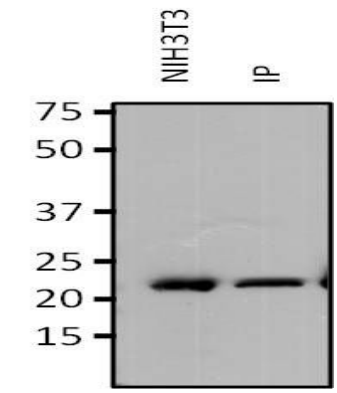
RAP1A Antibody (MA1-013) in ICC/IF

Immunofluorescent analysis of Rap1 (green) in HeLa cells. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% BSA/TBST (Product # 37525) for 15 minutes at room temperature. Cells were probed with a Rap1 monoclonal antibody (Product # MA1-013) at a dilution of 1:100 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488-conjugated goat anti-mouse IgG secondary antibody (Product # 35502) at a dilution of 1:400 for 30 minutes at room temperature. Nuclei (blue) were stained with Hoechst 33342 dye (Product # 62249). Images were taken on a Thermo Scientific ArrayScan at 20X magnification.



RAP1A Antibody (MA1-013) in IHC (P)

Immunohistochemistry analysis of Rap1 showing staining in the membrane of paraffin-embedded human kidney tissue (right) compared with a negative control without 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 Rap1 monoclonal antibody (Product # MA1-013) diluted in 3% BSA-PBS at a dilution of 1:200 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.



RAP1A Antibody (MA1-013) in IP

Immunoprecipitation of Rap1 was performed on NIH3T3 cells. Antigen-antibody complexes were formed by incubating 750 µg of NIH3T3 cell lysate with 2 µg of a Rap1 monoclonal antibody (Product # MA1-013) overnight on a rocking platform at 4°C. The immune complexes were captured on 50 µL Protein A/G Plus Agarose (Product # 20423), washed extensively, and eluted with 5X Lane Marker Reducing Sample Buffer (Product # 39000). Eluted sample (right lane) and 25 µg of NIH3T3 control lysate (left lane) were resolved on a 4-20% Tris-HCl polyacrylamide gel, transferred to a PVDF membrane, and blocked with 5% BSA/TBS-0.1%Tween for at least 1 hour. The membrane was probed with a Rap1 monoclonal antibody (Product # MA1-013) at a dilution of 1:500 overnight rotating at 4°C, washed in TBST, and probed with Clean-blot IP Detection Reagent (Product # 21230) at a dilution of 1:2500 for at least 1 hour. Chemiluminescent detection was performed using SuperSignal West Dura (Product # 34075).

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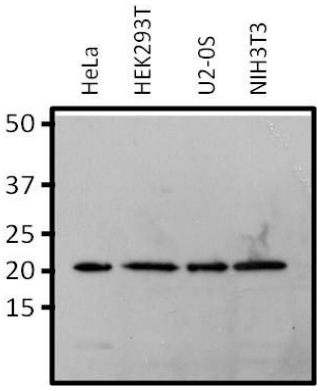

RAP1A Antibody (MA1-013) in WB

Western blot analysis of Rap1 was performed by loading 25 µg of various whole cell lysates per well onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with 5% BSA/TBST for at least 1 hour. The membrane was probed with a Rap1 monoclonal antibody (Product # MA1-013) at a dilution of 1:500 overnight at 4C on a rocking platform, washed in TBS-0.1%Tween-20, and probed with a goat anti-mouse IgG-HRP secondary antibody (Product # 32430) at a dilution of 1:15,000 for at least 1 hour. Chemiluminescent detection was performed using SuperSignal West Dura (Product # 34075).

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PubMed References For RAP1A Monoclonal Antibody (1D2-1C64)

1 Western Blot References

Species / Dilution	Summary
	MA1-013 was used in Western Blotting to conclude that in TJ-forming HDMECs, ArhGEF12 selectively activates Rap1A to limit capillary barrier disruption in a mechanism independent of cAMP-mediated Epac1 activation.
Human / Not Cited	FASEB journal : official publication of the Federation of American Societies for Experimental Biology (2022; 36:) **"ArhGEF12 activates Rap1A and not RhoA in human dermal microvascular endothelial cells to reduce tumor necrosis factor-induced leak."** Author(s):Khan A,Ni W,Baltazar T,Lopez-Giraldez F,Pober JS,Pierce RW PubMed Article URL:http://dx.doi.org/10.1096/fj.202101873RR

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