abcam

Product datasheet

Anti-RAC1 + Cdc42 (phospho S71) antibody ab5482

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Overview

Product name	Anti-RAC1 + Cdc42 (phospho S71) antibody
Description	Rabbit polyclonal to RAC1 + Cdc42 (phospho S71)
Host species	Rabbit
Specificity	Cdc 42 [pS71] (100% homologous) and Rho A/B/C [pS73] (92% homologous) are expected to react.
Tested applications	**Suitable for:** IHC-P, WB
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide corresponding to Human RAC1 + Cdc42 (phospho S71). The sequence is conserved in human and mouse RAC 1, 2, and 3, and Cdc 42 human, mouse, rat, dog and frog.
Positive control	WB: A431 cells treated with EGF. IHC-P: human skin, human stomach tissues
General notes	RAC, Cdc 42 and Rho A, B, and C are members of a small RhoGTPase family that bind and hydrolyze GTP. GTP bound RAC 1 and cdc 42 play a pivotal role in controlling cell shape, adhesion, growth and transformation. Active Rac 1 is implicated in regulating serum response element (SRE), NFAT 1 and nuclear factor kappa B (NF kappa B) transcription activities. Activated RAC 1 and Cdc 42 bind and activate PAK 1, which in turn activates key downstream signaling proteins including MEKK 1 and JNK. RAC 1 and Cdc 42 are phosphorylated on serine 71, a putative Akt site located between the protein binding domain and GTP binding domain. Phosphorylation of RAC 1 on serine 71 regulates its GTP binding and GTPase activity. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw

	cycles.
Storage buffer	pH: 7.30 Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.1% BSA BSA is IgG and protease free
Purity	Immunogen affinity purified
Purification notes	The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated RAC 1. The final product is generated by affinity chromatography using a RAC 1 derived peptide that is phosphorylated at serine 71.
Primary antibody notes	RAC, Cdc 42 and Rho A, B, and C are members of a small RhoGTPase family that bind and hydrolyze GTP. GTP bound RAC 1 and cdc 42 play a pivotal role in controlling cell shape, adhesion, growth and transformation. Active Rac 1 is implicated in regulating serum response element (SRE), NFAT 1 and nuclear factor kappa B (NF kappa B) transcription activities. Activated RAC 1 and Cdc 42 bind and activate PAK 1, which in turn activates key downstream signaling proteins including MEKK 1 and JNK. RAC 1 and Cdc 42 are phosphorylated on serine 71, a putative Akt site located between the protein binding domain and GTP binding domain. Phosphorylation of RAC 1 on serine 71 regulates its GTP binding and GTPase activity.
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab5482 in the following tested applications.

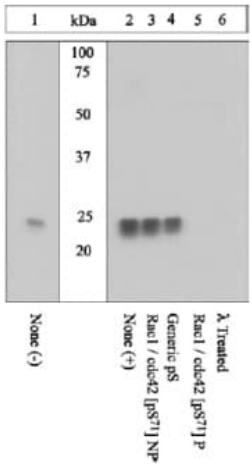
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		1/20 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB	★☆☆☆☆ **(1)**	1/1000. Detects a band of approximately 23 kDa.

Target

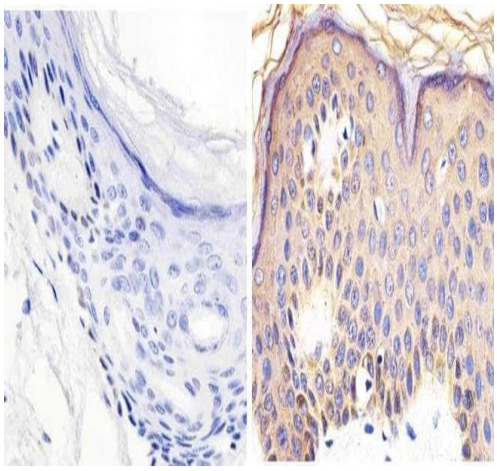
Relevance	Cdc42/Rac belongs to the superfamily of small GTPases that are structurally linked to the proto-oncogene product p21ras and are important for the control of cell growth and differentiation as well as for intracellular organization. Cdc42/Rac is an important upstream regulator of the protein kinase cascade that controls the SAPK/JNK and p38 activity. Recent data also suggest that constitutive active forms of Cdc42 can induce apoptosis through a mechanism requiring signaling through SAPK/JNK.
Cellular localization	Cell membrane; Lipid anchor; Cytoplasmic side.

Images



Western blot - Anti-RAC1 + Cdc42 (phospho S71) antibody (ab5482)

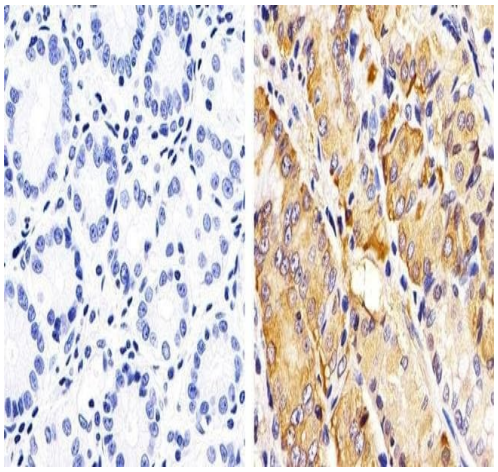
Peptide Competition and Phosphatase Treatment:
Lysates prepared from A431 cells left unstimulated (1) or stimulated with EGF (2-6) were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF. Membranes were either not treated (1-5) or treated with lambda phosphatase (6), blocked with a 5% BSA-TBST buffer for one hour at room temperature, and incubated with ab5482 antibody for two hours at room temperature in a 3% BSATBST buffer, following prior incubation with: no peptide (1, 2, 6), the nonphosphopeptide corresponding to the immunogen (3), a generic phosphoserine containing peptide (4), or, the phosphopeptide immunogen (5), After washing, membranes were incubated with goat F(ab')2 anti-rabbit IgG HRP conjugate and bands were detected using the Pierce SuperSignal method. The data show that the peptide corresponding to ab5482 blocks the antibody signal. The data also shows that phosphatase stripping eliminates the signal, verifying that the antibody is pho



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAC1 + Cdc42 (phospho S71) antibody (ab5482)

Immunohistochemiscal analysis of paraffin-embedded human skin tissue labeling RAC1 + Cdc42 (phospho S71) with ab5482 at 1/100 dilution (right) compared to 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 ab5482 diluted in 3% BSA-PBS at a dilution of 1/100 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAC1 + Cdc42 (phospho S71) antibody (ab5482)

Immunohistochemical analysis of paraffin-embedded human stomach tissue labeling RAC1 + Cdc42 (phospho S71) with ab5482 at 1/20 dilution (right) compared to 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 ab5482 diluted in 3% BSA-PBS at a dilution of 1/20 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.

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