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Anti-c-RAF (Phospho-S621) Antibody

Catalog # AP61146

Product Information

Application	WB, IHC, IF/IC
Primary Accession	P04049
Reactivity	Human, Mouse, Rat, Chicken, Bovine
Host	Rabbit
Clonality	Polyclonal
Calculated MW	73052

Additional Information

Gene ID	5894
Other Names	RAF; RAF proto-oncogene serine/threonine-protein kinase; Proto-oncogene c-RAF; cRaf; Raf-1
Target/Specificity	KLH-conjugated synthetic phosphopeptide corresponding to residues surrounding S621 of human c-RAF protein. The exact sequence is proprietary.
Dilution	WB~~WB (1/500 - 1/1000), IHC (1/50 - 1/200), IF/IC (1/100 - 1/500) IHC~~WB (1/500 - 1/1000), IHC (1/50 - 1/200), IF/IC (1/100 - 1/500) IF/IC~~N/A
Format	Liquid in 0.42% Potassium phosphate, 0.87% Sodium chloride, pH 7.3, 30% glycerol, and 0.01% sodium azide.
Storage	Store at -20 °C.Stable for 12 months from date of receipt

Protein Information

Name	RAF1 (HGNC:9829)
Synonyms	RAF
Function	Serine/threonine-protein kinase that acts as a regulatory link between the membrane-associated Ras GTPases and the MAPK/ERK cascade, and this critical regulatory link functions as a switch determining cell fate decisions including proliferation, differentiation, apoptosis, survival and oncogenic transformation. RAF1 activation initiates a mitogen-activated protein kinase (MAPK) cascade that comprises a sequential phosphorylation of the dual-specific MAPK kinases (MAP2K1/MEK1 and MAP2K2/MEK2) and the extracellular signal- regulated kinases (MAPK3/ERK1 and MAPK1/ERK2). The phosphorylated form of RAF1 (on residues Ser-338 and Ser-339, by PAK1) phosphorylates BAD/Bcl2-antagonist of cell death at 'Ser-75'. Phosphorylates adenylyl cyclases: ADCY2, ADCY5 and ADCY6, resulting in their activation. Phosphorylates PPP1R12A resulting in inhibition of the phosphatase activity.

Phosphorylates TNNT2/cardiac muscle troponin T. Can promote NF-kB activation and inhibit signal transducers involved in motility (ROCK2), apoptosis (MAP3K5/ASK1 and STK3/MST2), proliferation and angiogenesis (RB1). Can protect cells from apoptosis also by translocating to the mitochondria where it binds BCL2 and displaces BAD/Bcl2-antagonist of cell death. Regulates Rho signaling and migration, and is required for normal wound healing. Plays a role in the oncogenic transformation of epithelial cells via repression of the TJ protein, occludin (OCLN) by inducing the up-regulation of a transcriptional repressor SNAI2/SLUG, which induces down-regulation of OCLN. Restricts caspase activation in response to selected stimuli, notably Fas stimulation, pathogen-mediated macrophage apoptosis, and erythroid differentiation.

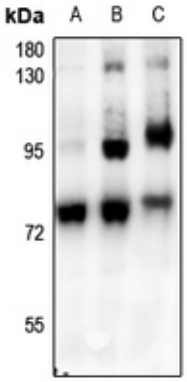
Cellular Location

Cytoplasm. Cell membrane. Mitochondrion. Nucleus. Note=Colocalizes with RGS14 and BRAF in both the cytoplasm and membranes. Phosphorylation at Ser-259 impairs its membrane accumulation. Recruited to the cell membrane by the active Ras protein Phosphorylation at Ser-338 and Ser-339 by PAK1 is required for its mitochondrial localization. Retinoic acid-induced Ser-621 phosphorylated form of RAF1 is predominantly localized at the nucleus

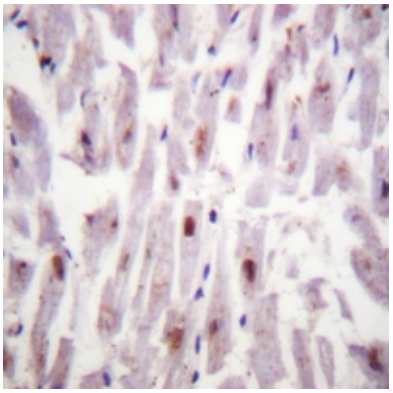
Tissue Location

In skeletal muscle, isoform 1 is more abundant than isoform 2.

Images

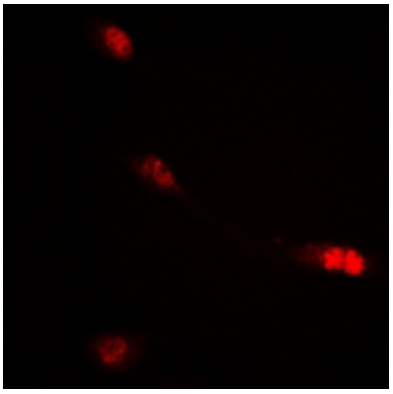


Western blot analysis of c-RAF (Phospho-S621) expression in Panc1 (A), HEK293T (B), PC3 (C) whole cell lysates. (Predicted band size: 73 kD; Observed band size: 74 kD)

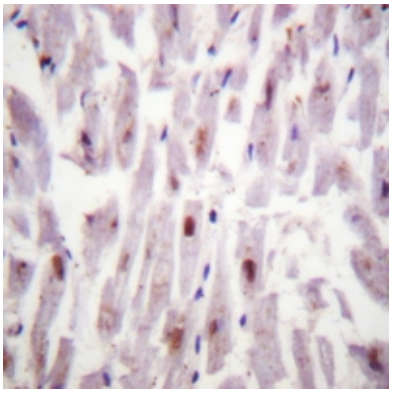


Immunohistochemical analysis of c-RAF (Phospho-S621) staining in human heart formalin fixed paraffin embedded tissue section. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0). The section was then incubated with the antibody at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

Immunofluorescent analysis of c-RAF (Phospho-S621) staining in HeLa cells. 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 the primary antibody in 3% BSA-PBS and incubated overnight at 4 °C in a hidified chamber. Cells were washed with PBST and incubated with a Alexa Fluor 594-conjugated secondary



antibody (red) in PBS at room temperature in the dark.



Immunohistochemical analysis of c-RAF (pS621) staining in human heart formalin fixed paraffin embedded tissue section. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0). The section was then incubated with the antibody at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

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