

# CPTC-RAF1-8 (CAB080387)

**Uniprot ID:** P04049

**Protein name:** RAF1\_HUMAN

**Full name:** RAF proto-oncogene serine/threonine-protein kinase

**Tissue specificity:** In skeletal muscle, isoform 1 is more abundant than isoform 2.

**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- $\kappa$ B 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.

**Subcellular location:**

Cytoplasm

Cell membrane

Mitochondrion

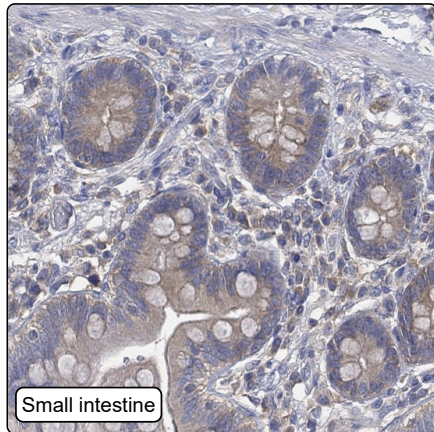
Nucleus

**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.

**Protein existence:** Experimental evidence at protein level

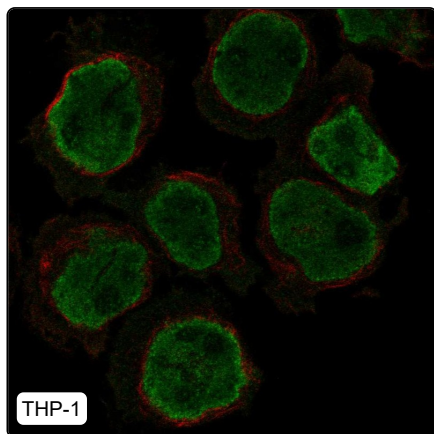
**Comment:** IF: Ok for both cell lines in IF/Charlotte

## Immunohistochemistry



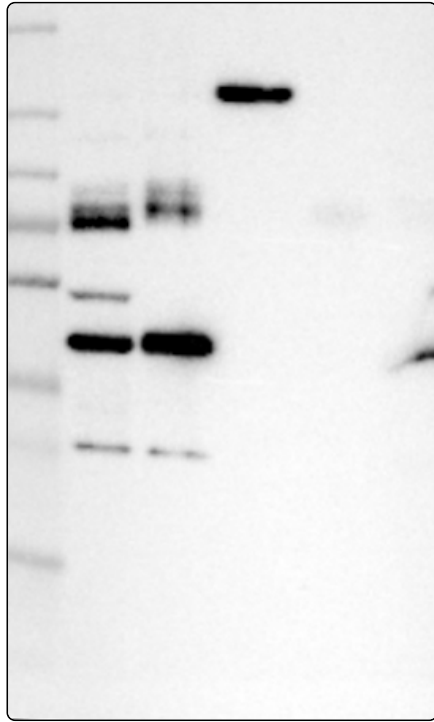
<b>IHC protocol:</b>	HIER pH6, Dilution 1:400
<b>IHC test staining:</b>	Cytoplasmic positivity in gastrointestinal tract.
<b>Literature conformance:</b>	Not consistent with gene/protein characterization data
<b>Literature significance:</b>	
<b>RNA similarity:</b>	Very low consistency between antibody staining and RNA expression data
<b>RNA tissue specificity:</b>	Low tissue specificity
<b>RNA tissue distribution:</b>	Detected in all
<b>IHC Sibling similarity:</b>	Other antibody shows dissimilar IHC staining pattern

## Immunofluorescence



<b>IF Overlay:</b>	antibody (green), anti-tubulin (red) and DAPI (blue)
<b>IF main location:</b>	Nucleoplasm - 3: <b>Supportive</b> (auto)
<b>IF additional location:</b>	
<b>IF approved for publication on HPA:</b>	No
<b>IF in THP-1:</b>	Nucleoplasm
<b>IF in U2OS:</b>	Nucleoplasm

# Western blot



<b>WB Size markers (kDa):</b>	250, 130, 100, 70, 55, 35, 25, 15, 10
<b>WB Lanes:</b>	Marker (1), RT-4 (2), U-251MG (3), Plasma (4), Liver (5), Tonsil (6)
<b>WB Target weight (kDa):</b>	60, 73, 75
<b>WB Validation:</b>	Supported (Band of predicted size in kDa (+/-20%) with additional bands present.)