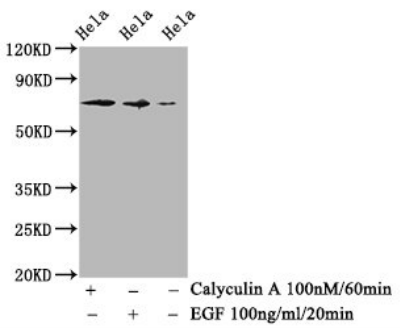
Phospho-RAF1 (S259) Recombinant Monoclonal Antibody

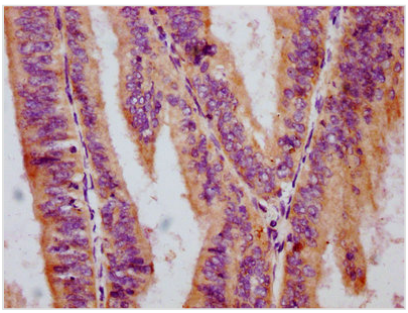
Product Code	CSB-RA019284A259phHU
Abbreviation	RAF proto-oncogene serine/threonine-protein kinase
Storage	Upon receipt, store at -20°C or -80°C. Avoid repeated freeze.
Uniprot No.	P04049
Immunogen	A synthesized peptide derived from Human Phospho-RAF1 (S259)
Species Reactivity	Human
Tested Applications	ELISA, WB, IHC, IF; Recommended dilution: WB:1:500-1:5000, IHC:1:50-1:200, IF:1:20-1:200
Relevance	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.
Form	Liquid
Conjugate	Non-conjugated
Storage Buffer	Rabbit IgG in phosphate buffered saline , pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol.
Purification Method	Affinity-chromatography
Isotype	Rabbit IgG
Clonality	Monoclonal

Alias	RAF proto-oncogene serine/threonine-protein kinase, Proto-oncogene c-RAF, cRaf, Raf-1, RAF1, RAF
Immunogen Species	Homo sapiens (Human)
Research Area	Signal Transduction
Gene Names	RAF1
Clone No.	4C9

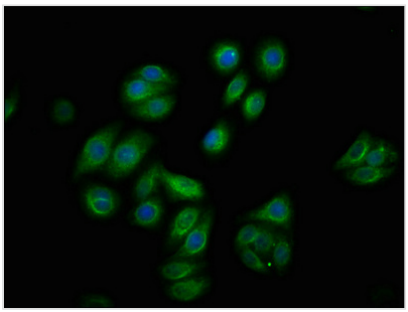
Image



Western Blot
Positive WB detected in Hela whole cell lysate(treated with Calyculin A or EGF)
All lanes Phospho-RAF1 antibody at 1.18µg/ml
Secondary
Goat polyclonal to rabbit IgG at 1/50000 dilution
Predicted band size: 73 KDa
Observed band size: 73 KDa



IHC image of CSB-RA019284A259phHU diluted at 1:100 and staining in paraffin-embedded human endometrial cancer performed on a Leica BondTM system. After dewaxing and hydration, antigen retrieval was mediated by high pressure in a citrate buffer (pH 6.0). Section was blocked with 10% normal goat serum 30min at RT. Then primary antibody (1% BSA) was incubated at 4? overnight. The primary is detected by a biotinylated secondary antibody and visualized using an HRP conjugated SP system.



Immunofluorescence staining of HepG2 cells with CSB-RA019284A259phHU at 1:100,counter-stained with DAPI. The cells were fixed in 4% formaldehyde, permeabilized using 0.2% Triton X-100 and blocked in 10% normal Goat Serum. The cells were then incubated with the antibody overnight at 4?. The secondary antibody was Alexa Fluor 488-congugated AffiniPure Goat Anti-Rabbit IgG (H+L).

Description

To create the phospho-RAF1 (S259) recombinant monoclonal antibody, the first step involves obtaining the genes responsible for coding this RAF1 antibody. These genes are sourced from rabbits previously exposed to a synthesized peptide derived from the human RAF1 protein phosphorylated at S259. Subsequently, these antibody genes are cloned into specialized expression vectors. Following this genetic modification, the vectors are carefully transfected into host suspension cells, which are then diligently cultivated to promote the expression and secretion of antibodies. The phospho-RAF1 (S259) recombinant monoclonal antibody is then subjected to a meticulous purification process using affinity chromatography, effectively separating the antibody from the surrounding

cell culture supernatant. Finally, the antibody's functionality is rigorously assessed through a battery of tests, including ELISA, WB, IHC, and IF, confirming its capability to effectively interact with the human RAF1 protein phosphorylated at S259.

Phosphorylation of RAF1 at S259 is a critical regulatory event in the MAPK signaling pathway, helping to control cell proliferation, differentiation, and survival. Dysregulation of this phosphorylation event can have significant implications for cancer and other diseases driven by abnormal signaling pathways.