B-Raf Antibody
Cat. No. CP10027

Applications:	**WB, IP, IHC, ICC**
Detected MW:	**94 kDa**
Species & Reactivity:	**Human, Mouse**
Isotype:	**Mouse IgG1**

BACKGROUND

The RAF protein kinases family contains three serine/threonine kinases including A-RAF, B-RAF, and C-RAF. They share three conserved regions: CR1 and CR2 within the regulatory NH_2 terminus and CR3 encompassing the kinase domain within the COOH terminus. They are part of the ras-MAPK signaling cascade and phosphorylate MEK.[1] RAF proteins are normally cytosolic but they are recruited to the plasma membrane by the small G-protein RAS, and this is an essential step for their activation by growth factors, cytokines, and hormones. At the membrane, RAF activation occurs through a highly complex process involving conformation changes, binding to other proteins, binding to lipids, and phosphorylation and dephosphorylation of some residues. This activation process is tightly regulated by a number of factors including phosphatases (e.g. PP1, PP2A,PP5), kinases (e.g. Src, ERK, Akt, PKC) and proteins that bind directly to Raf-1 (e.g. RKIP, 14-3-3zeta, KSR, Hsp90). Within the kinase domain, phosphorylation of two motifs is required for activation. One of these is called the activation segment, which must be phosphorylated on conserved threonine and serine residues (In B-RAF, these are T599 and S602, and in C-RAF, the corresponding residues are T491 and S494). The other motif that must be phosphorylated is called the negative-charge regulatory or N-region. In C-RAF, the N-region sequence is ^{338}SSY^{341}Y and phosphorylation of S338 and Y341 is essential for activation by RAS and growth factors. Both sites are conserved in A-RAF (S299 and T302, respectively) but in B-RAF, Y340 and Y341 are replaced by aspartic acids (D448 and D449) and although S338 is conserved, it is constitutively phosphorylated. All RAF isoforms are only fully activated when four negative charges occupy the region.[2] Thr401 phosphorylation was induced by PDGF stimulation.[3]

B-RAf is commonly mutated and thereby activated in many human cancers, the most frequent mutation being the V600E mutation of the kinase domain. Whilst wt B-RAF and Raf-1 are strongly activated by growth factor signals via Ras and Src, A-RAF is only modestly activated and has low basal activity. All three isoforms of Raf are considered to be oncogenic.[4]

References:

1. Wan, P.T. et al: Cell 116:855-67, 2004
2. Morrison, D.K. & Cutler, Jr, R.E.: Curr. Opin. Cell Biol. 9:174-9, 1997
3. Ritt, D.A. et al: Mol. Cell Biol. 30:806-19, 2010
4. Dhillon, A.S. & Kolch, W.: Cancer Cell 5:303-4, 2004

TECHNICAL INFORMATION

Source:
B-Raf Antibody is a mouse monoclonal antibody raised against purified recombinant human B-RAF protein fragments expressed in *E. coli*.

Specificity and Sensitivity:
This antibody detects endogenous B-RAF proteins without cross-reactivity with other family members.

Storage Buffer: PBS and 30% glycerol

Storage:
Store at -20°C for at least one year. Store at 4°C for frequent use. Avoid repeated freeze-thaw cycles.

APPLICATIONS

Application:	*Dilution:
WB	1:500-2000
IP	1:50
IHC	1:200-1000
ICC	1:200-1000
FACS	n/d

**Optimal dilutions must be determined by end user.*

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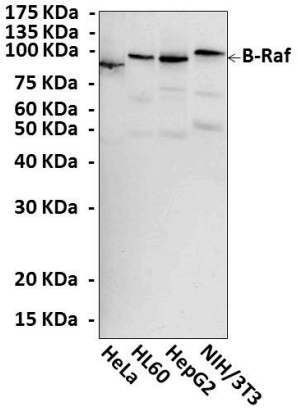
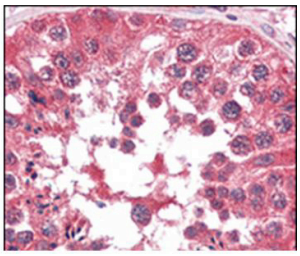
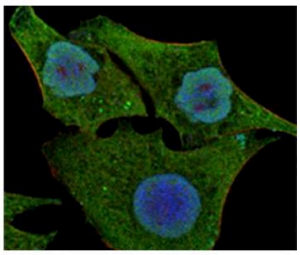
QUALITY CONTROL DATA







Top: Western Blot detection of B-RAF proteins various cell lysates using B-RAF Antibody. **Middle:** This antibody stains paraffin-embedded human testis tissue in immunohistochemical analysis. **Bottom:** It also stains MCF7 cells in confocal immunofluorescent testing.