Technical Data Sheet

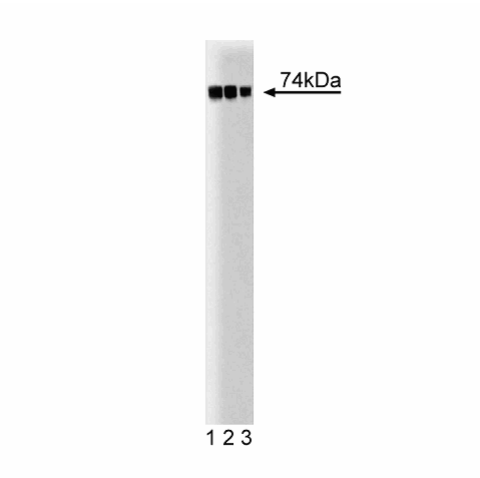
Purified Mouse Anti-c-Raf

Product Information

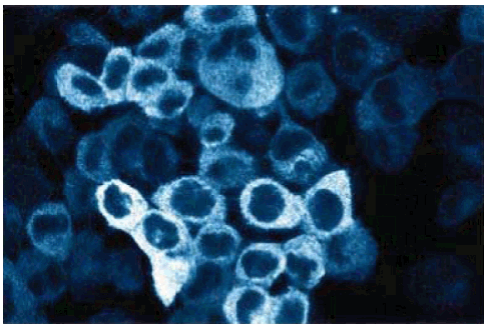
Material Number:	**610152**
Size:	150 µg
Concentration:	250 µg/ml
Clone:	53/c-Raf-1
Immunogen:	Human c-Raf-1 aa. 162-378
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Human Tested in Development: Dog, Rat, Mouse, Chicken
Target MW:	74 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

Raf (c-Raf-1) is a cytoplasmic serine/threonine protein kinase and is a member of a family of proteins which are highly conserved from *Drosophila* to mammals. Raf has a critical role in the response to many growth factors including: EGF, PDGF, insulin, IL-2, IL-3, CSF-1, and GM-CSF. Raf can directly interact with Ras-GTP and subsequently become activated. Thus Raf plays a prominent role in the Ras signaling pathway by transferring a signal from Ras which has been activated by growth factor receptor-stimulated tyrosine kinase activity. This leads to the stimulation and activation of a number of other cytoplasmic serine/threonine kinases. Raf regulates the MAP kinase pathway by phosphorylating and activating MEK, which then phosphorylates and activates MAP kinase (ERK). ERK then phosphorylates and activates Rsk. Raf activity can also be regulated independently of Ras. Cyclic AMP (cAMP) activation of protein kinase A (PKA) can inhibit growth factor stimulation of Raf.



Western blot analysis of c-Raf on A431 lysate. *Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of c-Raf antibody.*



Immunofluorescent staining of A431 cells.

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at -20°C.

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Application Notes

Application

Western blot	Routinely Tested
Immunoprecipitation	Tested During Development
Immunofluorescence	Tested During Development
Immunohistochemistry	Not Recommended

Recommended Assay Procedure:

Western blot: Please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml .

Suggested Companion Products

Catalog Number	Name	Size	Clone
611447	A431 Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Bondzi C, Grant S, Krystal GW. A novel assay for the measurement of Raf-1 kinase activity. *Oncogene.* 2000; 19(43):5030-5033.(Clone-specific: Immunoprecipitation, In vitro kinase assay, Western blot)

Cheng JJ, Wung BS, Chao YJ, Wang DL. Sequential activation of protein kinase C (PKC)-alpha and PKC-epsilon contributes to sustained Raf/ERK1/2 activation in endothelial cells under mechanical strain. *J Biol Chem.* 2001; 276(33):31368-31375.(Clone-specific: Immunoprecipitation, Western blot)

Coles LC, Shaw PE. PAK1 primes MEK1 for phosphorylation by Raf-1 kinase during cross-cascade activation of the ERK pathway. *Oncogene.* 2002; 21(14):2236-2244.(Clone-specific: Immunoprecipitation, Western blot)

Rapp UR. Role of Raf-1 serine/threonine protein kinase in growth factor signal transduction. *Oncogene.* 1991; 6(4):495-500.(Biology)

Rizzo MA, Kraft CA, Watkins SC, Levitan ES, Romero G. Agonist-dependent traffic of raft-associated Ras and Raf-1 is required for activation of the mitogen-activated protein kinase cascade. *J Immunol.* 2001; 276(37):34928-34933.(Clone-specific: Immunofluorescence, Western blot)