

C-Raf (Y340D; Y341D) [GST-tagged]

Kinase

Alternate Names: RAF proto-oncogene serine/threonine-protein kinase, Proto-oncogene c-RAF, RAF1

Cat. No. 66-0024-050

Lot. No. 30303

Quantity: 50 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 1 of 2

Background

There are three Raf kinase family members, all serine/threonine kinases, identified as: A-Raf, B-Raf and C-Raf (Rahman *et al.*, 2013). C-Raf 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 (Chen *et al.*, 2001). Cloning of the C-Raf gene was first described by Rapp *et al.* (1983). Regulation is a highly complex process involving membrane recruitment, protein-protein interactions, dimerization, and phosphorylation/dephosphorylation events. Ras-GTP recruits C-Raf to the membrane, thereby promoting its activation. The inactive conformation of C-Raf is maintained by auto-inhibitory interactions occurring between the N-terminal regulatory and the C-terminal catalytic domains and by the binding of a 14-3-3 protein that contacts two phosphorylation sites on the kinase (Abraham *et al.*, 2000). Although C-Raf is capable of mutating into an oncogene in experimental settings, it is B-Raf that is the true major player in carcinogenesis in humans. Approximately 20% of all examined human tumour samples display a mutated B-Raf gene (Emuss *et al.*, 2005). The overwhelming majority of these mutations involve the exchange of a single amino acid (V600E) that can mimic the activation loop phosphorylation and immediately render the ki-

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Physical Characteristics

Species: human

Source: baculovirus expression vector system

Quantity: 50 µg

Concentration: 0.48 mg/ml

Formulation: 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 150 mM NaCl, 0.1% β-Mercaptoethanol, 270 mM sucrose, 0.03% Brij-35, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: ~65.8 kDa

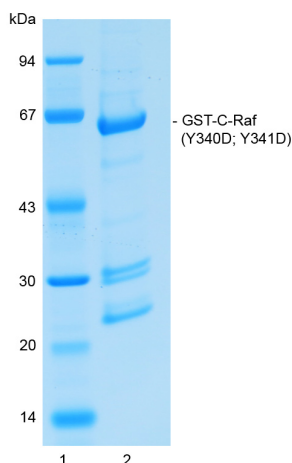
Purity: >80% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70°C; aliquot as required

Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 2.5 µg GST-C-Raf
(Y340D; Y341D)



Protein Identification:
Confirmed by mass spectrometry.

Activity Assay:
The specific activity of GST-C-Raf (Y340D; Y341D) was determined using the method described by Hastie *et al.* (2006) with the enzyme being assayed at several concentrations. Initially, GST-C-Raf (Y340D; Y341D) (diluted in 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 1 mg/ml BSA, 10 mM DTT) was incubated with MKK1 (0.4 µg), p42MAPK (0.4 µg) and ATP (0.1 mM) in 50 mM Tris/HCl pH7.5, 0.1 mM EGTA, 10 mM MgAc, 10 mM DTT buffer for 30 minutes at 30°C. A sample of this GST-C-Raf (Y340D; Y341D) reaction was then incubated for 10 minutes at 30°C in kinase reaction buffer in the presence of MBP substrate (0.33 mg/ml) and [γ-32P]ATP (100 µM). Duplicate reactions were stopped by spotting the assay mixture onto Whatman P81 paper – capturing the phosphorylated substrate. The radioactivity incorporated was measured on a scintillation counter and the enzyme's mean specific activity was calculated.

GST-C-Raf (Y340D; Y341D) specific activity:
910028 Units/mg (436813 Units/ml)

1 Unit = 1 nmole of phosphate incorporated into the substrate in 1 minute

Substrate: Myelin Basic Protein (MBP)



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Lot-specific COA version tracker: v1.0.0

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CERTIFICATE OF ANALYSIS Page 2 of 2

Background

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nase domain fully active (Tran *et al.*, 2005). Since B-Raf can also activate itself by homodimerisation and C-Raf by heterodimerisation, this mutation has a catastrophic effect by turning the ERK1/2 pathway constitutively active, driving an uncontrolled process of cell division (Garnett *et al.*, 2005). Genetic mutations Y340D and Y341D have been shown to achieve maximum activation of C-Raf, simulating tyrosine phosphorylation and recruitment to the plasma membrane (Roy *et al.*, 1997).

References:

Abraham D, Podar K, Pacher M, Kubicek M, Welzel N, Hemmings BA, *et al.* (2000) Raf-1-associated protein phosphatase 2A as a positive regulator of kinase activation. *J Biol Chem* **275**, 22300-22304.

Chen J, Fujii K, Zhang L, Roberts T and Fu H (2001) Raf-1 promotes cell survival by antagonizing apoptosis signal-regulating kinase 1 through a MEK-ERK independent mechanism. *Proc Natl Acad Sci U S A* **98**, 7783-7788.

Emuss V, Garnett M, Mason C and Marais R (2005) Mutations of C-RAF are rare in human cancer because C-RAF has a low basal kinase activity compared with B-RAF. *Cancer Res* **65**, 9719-9726.

Garnett MJ, Rana S, Paterson H, Barford D and Marais R (2005) Wild-type and mutant B-RAF activate C-RAF through distinct mechanisms involving heterodimerization. *Mol Cell* **20**, 963-969.

Hastie CJ, McLauchlan HJ, Cohen P (2006) Assay of protein kinases using radiolabeled ATP: a protocol. *Nat Protoc* **1**, 968-71.

Rahman MA, Salajegheh A, Smith RA and Lam AK (2013) B-Raf mutation: a key player in molecular biology of cancer. *Exp Mol Pathol* **95**, 336-342.

Rapp UR, Goldsborough MD, Mark GE, Bonner TI, Groffen J, Reynolds FH, Jr., *et al.* (1983) Structure and biological activity of v-raf, a unique oncogene transduced by a retrovirus. *Proc Natl Acad Sci U S A* **80**, 4218-4222.

Roy S, Lane A, Yan J, McPherson R and Hancock JF (1997) Activity of plasma membrane-recruited Raf-1 is regulated by Ras via the Raf zinc finger. *J Biol Chem* **272**, 20139-20145.

Tran NH, Wu X and Frost JA (2005) B-Raf and Raf-1 are regulated by distinct autoregulatory mechanisms. *J Biol Chem* **280**, 16244-16253.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSR IAYS KDFETLKVDFL
SKLP EMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAI PQIDKYLKSSKYIAWPLQGWQAT
FGGGDHPPKSDLEVL FQGPLGS SQPKTPV
PAQRERAPVSGTQEK NKIRPRGQRDSSD
DWEIEASEVMLSTRIGSGSFGTVYK GKWHGD
VAVKILKVVDP TPEQFQAFRNEVAVLRK
TRHVNILLFMGYMTKDNLAIVTQWCEGSS
LYKHLHVQETK FQMFQLIDIARQTAQGM
DYLHAKNI IHRDMKSNNIFLHEGLTVKIGD
FGLATVKS RWSGSQQVEQPTGSVLWMAPE
VIRMQDNNPFSFQSDVYSYGIVLYELMT
GELPYSHINNRDQIIFMVGRGYASPDLSK
LYKNC PKAMKRLVADCVKVKKEERPLFPQ
ILSSI ELLQHSLPKINRSASEPSLHRAHTED
INACTLTTSRPLPVF

Tag (**bold text**): N-terminal GST

Protease cleavage site: PreScission™ (LEVL FQ▼GP)

C-Raf (regular text): Start **bold italics** (amino acid residues 306-648).

The enzyme has two mutations (Y340D and Y341D) to mimic phosphorylation and activation of C-Raf

Accession number: NP_002871



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