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**	Quantity:	50 µg
Lot. No.	**30303**	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 autoinhibitory 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% ß-Mercaptoethanol, 270 mM sucrose, 0.03% Brij-35, 1 mM Benzamidine, 0.2 mM PMSF

Molecular Weight: ~65.8 kDa

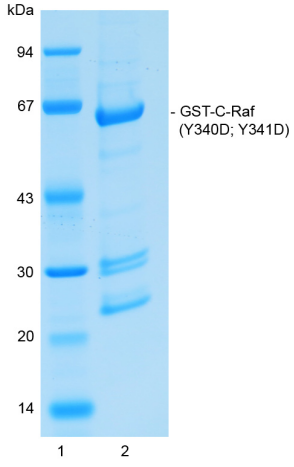
Purity: >80% by InstantBlue™ SDS-PAGE

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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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Kinase

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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.

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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
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQAT
FGGGDHPPKSDLEVLFQGPLGS***S***QPKTPV
PAQRERAPVSGTQEKNKIRPRGQRDSSD
DWEIEASEVMLSTRIGSGSFGTVYKGKWHGD
VAVKILKVVDPTPEQFQAFRNEVAVLRK
TRHVNILLFMGYMTKDNLAIVTQWCEGSS
LYKHLHVQETKFQMFQLIDIARQTAQGM
DYLHAKNIIHRDMKSNNIFLHEGLTVKIGD
FGLATVKSRWSGSQQVEQPTGSVLWMAPE
VIRMQDNNPFSFQSDVYSYGIVLYELMT
GELPYSHINNRDQIIFMVGRGYASPDLSK
LYKNCPKAMKRLVADCVKKVKEERPLFPQ
ILSSIELLQHSLPKINRSASEPSLHRAAHTED
INACTLTTSPRLPVF

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼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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