Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active c-Raf Y340D, Y341D [306 - 648]

Enzyme description:- c-Raf Y340D, Y341D [306 – 648]

Clone number:- DU 811

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 0.1 mg/L

Calculated molecular mass:- 65, 727 daltons

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35

Storage temperature:- -70 °C

Assay:-

Three step assay in which c-Raf activates unactive MKK1 [DU 1843], which in turn activates unactive p42MAPKinase [DU 650 or DU 1844]. Activity of p42MAPkinase is then assayed against MBP as substrate (final concentration of 0.3 mg/ml), in the standard filter binding assay.

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol,
0.2 mM sodium vanadate, 0.5 µM microcystin-LR, 10 mM magnesium acetate

Specific activity range:- 250, 000 - 500, 000 U/mg

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Clone Data Sheet

c-Raf Y340D, Y341D [306 – 648]

Protein c-Raf Y340D Y341D [306 – 648]

Clone number DU 811

Species Human

Accession no BC018119

Tags N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**SQPKTPVPA**
QRERAPVSGTQEKNKIRPRGQRDSSDDWEIEASEVMLSTRIGSGSFGT
VYKGKWHGDVAVKILKVVDPTPEQFQAFRNEVAVLRKTRHVNILLFMG
YMTKDNLAIVTQWCEGSSLYKHLHVQETKFQMFQLIDIARQTAQGMDY
LHAKNIIHRDMKSNNIFLHEGLTVKIGDFGLATVKSRWSGSQQVEQPT
GSVLWMAPEVIRMQDNNPFSFQSDVYSYGIVLYELMTGELPYSHINNR
DQIIFMVGRGYASPDLSKLYKNCPKAMKRLVADCVKKVKEERPLFPQI
LSSIELLQHSLPKINRSASEPSLHRAAHTEDINACTLTTSPRLPVF

Native sequence Amino acids S306 – F648 (end) of human c-Raf.
Residue S232 of the fusion protein is equivalent to S306 of the native enzyme. The enzyme has a Y430D and a Y341D mutation in order to mimic phosphorylation of the enzyme. Residues Y430 and Y431 are equilivalent to D266 and D267 of the fusion protein. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGPL) at residues 221 – 229

Cloning sites *Bam*HI and *Eco*RI site of pFastBAC GST

Nucleotide sequence of insert

GGATCCTCACAGCCGAAAACCCCCGTGCCAGCACAAAGAGAGCGGGCA
CCAGTATCTGGGACCCAGGAGAAAAACAAAATTAGGCCTCGTGGACAG
AGAGATTCAAGCGATGATTGGGAAATAGAAGCCAGTGAAGTGATGCTG
TCCACTCGGATTGGGTCAGGCTCTTTTGGAACTGTTTATAAGGGTAAA
TGGCACGGAGATGTTGCAGTAAAGATCCTAAAGGTTGTCGACCCAACC
CCAGAGCAATTCCAGGCCTTCAGGAATGAGGTGGCTGTTCTGCGCAAA
ACACGGCATGTGAACATTCTGCTTTTCATGGGGTACATGACAAAGGAC
AACCTGGCAATTGTGACCCAGTGGTGCGAGGGCAGCAGCCTCTACAAA
CACCTGCATGTCCAGGAGACCAAGTTTCAGATGTTCCAGCTAATTGAC
ATTGCCCGGCAGACGGCTCAGGGAATGGACTATTTGCATGCAAAGAAC
ATCATCCATAGAGACATGAAATCCAACAATATATTTCTCCATGAAGGC
TTAACAGTGAAAATTGGAGATTTTGGTTTGGCAACAGTAAAGTCACGC
TGGAGTGGTTCTCAGCAGGTTGAACAACCTACTGGCTCTGTCCTCTGG
ATGGCCCCAGAGGTGATCCGAATGCAGGATAACAACCCATTCAGTTTC
CAGTCGGATGTCTACTCCTATGGCATCGTATTGTATGAACTGATGACG
GGGGAGCTTCCTTATTCTCACATCAACAACCGAGATCAGATCATCTTC
ATGGTGGGCCGAGGATATGCCTCCCCAGATCTTAGTAAGCTATATAAG
AACTGCCCCAAAGCAATGAAGAGGCTGGTAGCTGACTGTGTGAAGAAA
GTAAAGGAAGAGAGGCCTCTTTTTCCCCAGATCCTGTCTTCCATTGAG
CTGCTCCAACACTCTCTACCGAAGATCAACCGGAGCGCTTCCGAGCCA
TCCTTGCATCGGGCAGCCCACACTGAGGATATCAATGCTTGCACGCTG
ACCACGTCCCCGAGGCTGCCTGTCTTCtaggaattc