Certificate of Analysis

REACTION BIOLOGY

ProQinase™ RAF1 Y340D/Y341D (GST-HIS-tag)

Raf-1 proto-oncogene, serine/threonine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: RAF1

Synonyms: cRAF, CRAF, NS5

Product No.: 0792-0000-1

Lot: 001

Description: Human RAF1 C-terminal fragment, amino acids S_{306}-F_{648} (as in NCBI/Protein entry NP_002871.1), Y340D and Y341D mutant, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: RAF1 Y340D/Y341D Lot 001 was confirmed as RAF1 by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 67,409 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

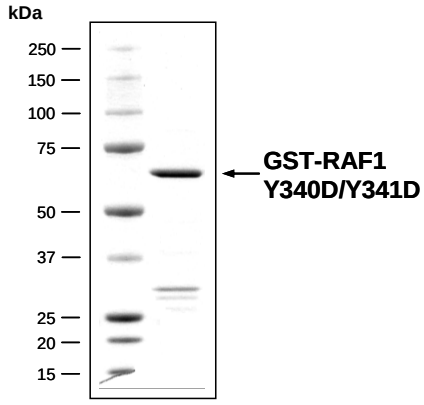
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.301 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

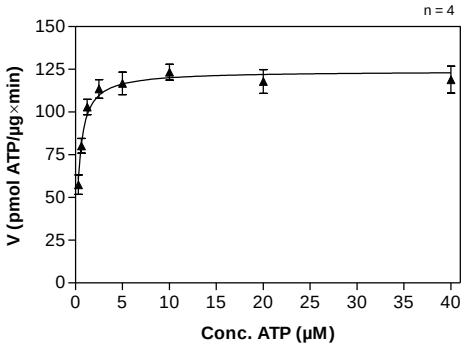
Biochemical Parameters:
Specific kinase activity (P_i transfer): 124 pmol/µg × min
ATP-K_M: 0.32 µM

RAF1 YDYD Lot 001:
Coomassie stain



2.0 µg GST-RAF1 Y340D/Y341D

RAF1 YDYD Lot 001:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: MEK1-KM, 40 µg/ml
  Kinase: 400 ng/ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology:
RAF1 YDYD Lot 001 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

Recombinant Proteins

ProQinase™ RAF1 Y340D/Y341D (GST-HIS-tag)

Product No.: 0792-0000-1

GST-RAF1 YDYD Recombinant Fusion Protein Amino Acid Sequence

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  1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID  60
 61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV 120
121  DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
241  PLAMGSQPKT PVPAQRERAP VSGTQEKNKI RPRGQRDSSD DWEIEASEVM LSTRIGSGSF 300
301  GTVYKGKWHG DVAVKILKVV DPTPEQFQAF RNEVAVLRKT RHVNILLFMG YMTKDNLAIV 360
361  TQWCEGSSLY KHLHVQETKF QMFQLIDIAR QTAQGMDYLH AKNIIHRDMK SNNIFLHEGL 420
421  TVKIGDFGLA TVKSRWSGSQ QVEQPTGSVL WMAPEVIRMQ DNNPFSFQSD VYSYGIVLYE 480
481  LMTGELPYSH INNRDQIIFM VGRGYASPDL SKLYKNCPKA MKRLVADCVK KVKEERPLFP 540
541  QILSSIELLQ HSLPKINRSA SEPSLHRAAH TEDINACTLT TSPRLPVF              600
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: RAF1 fragment **DD**: YDYD mutation

RAF1 wt[1] Amino Acid Sequence

```
  1  MEHIQGAWKT ISNGFGFKDA VFDGSSCISP TIVQQFGYQR RASDDGKLTD PSKTSNTIRV  60
 61  FLPNKQRTVV NVRNGMSLHD CLMKALKVRG LQPECCAVFR LLHEHKGKKA RLDWNTDAAS 120
121  LIGEELQVDF LDHVPLTTHN FARKTFLKLA FCDICQKFLL NGFRCQTCGY KFHEHCSTKV 180
181  PTMCVDWSNI RQLLLFPNST IGDSGVPALP SLTMRRMRES VSRMPVSSQH RYSTPHAFTF 240
241  NTSSPSSEGS LSQRQRSTST PNVHMVSTTL PVDSRMIEDA IRSHSESASP SALSSSPNNL 300
301  SPTGWSQPKT PVPAQRERAP VSGTQEKNKI RPRGQRDSSY YWEIEASEVM LSTRIGSGSF 360
361  GTVYKGKWHG DVAVKILKVV DPTPEQFQAF RNEVAVLRKT RHVNILLFMG YMTKDNLAIV 420
421  TQWCEGSSLY KHLHVQETKF QMFQLIDIAR QTAQGMDYLH AKNIIHRDMK SNNIFLHEGL 480
481  TVKIGDFGLA TVKSRWSGSQ QVEQPTGSVL WMAPEVIRMQ DNNPFSFQSD VYSYGIVLYE 540
541  LMTGELPYSH INNRDQIIFM VGRGYASPDL SKLYKNCPKA MKRLVADCVK KVKEERPLFP 600
600  QILSSIELLQ HSLPKINRSA SEPSLHRAAH TEDINACTLT TSPRLPVF              660
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blue: RAF1 sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_002871.1

Recombinant Proteins