

## 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<sub>306</sub>-F<sub>648</sub> (as in [NCBI/Protein](#) entry NP\_002871.1), Y340D and Y341D mutant, N-terminal GST-HIS<sub>6</sub> 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<sub>Fusion Protein</sub>:** 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)

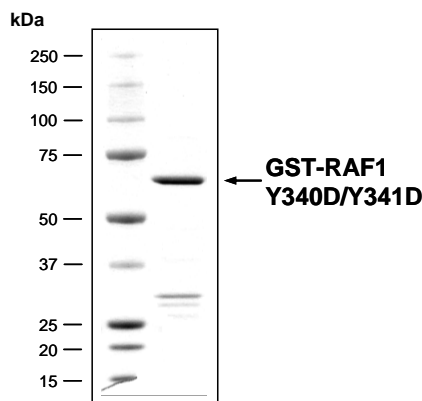
### Biochemical Parameters:

Specific kinase activity (P<sub>i</sub> transfer): 124 pmol/µg × min  
ATP-K<sub>M</sub>: 0.32 µM

### Additional assay technology:

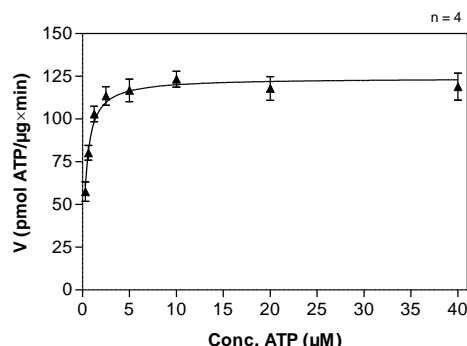
RAF1 YDYD Lot 001 was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega  
ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

### RAF1 YDYD Lot 001: Coomassie stain



2.0 µg GST-RAF1 Y340D/Y341D

### RAF1 YDYD Lot 001: Determination of V<sub>max</sub> and K<sub>M</sub> value for ATP



### Determination of K<sub>M</sub> value & Specific activity:

- Assay conditions:
  - 60 mM HEPES-NaOH, pH 7.5
  - 3 mM MgCl<sub>2</sub>
  - 3 mM MnCl<sub>2</sub>
  - 3 µM Na-orthovanadate
  - 1.2 mM DTT
  - 50 µg/ml PEG<sub>20,000</sub>
  - ATP (variable)
  - Substrate: MEK1-KM, 40 µg/ml
  - Kinase: 400 ng/ml
- Filter binding assay
- MSFC membrane (Millipore)

## RAF1 Y340D/Y341D (GST-HIS-tag)

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GST-RAF1 YDYD Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLIERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWAQTF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFGG	240
241	PLAMGSQPKT	PVPAQRERAP	VSGTQEKNKI	RPRGQRDSSD	DWEIEASEVM	LSTRIGSGSF	300
301	GTVYKGGKWHG	DVAVKILKVV	DPTPEQFQAF	RNEVAVLRKT	RHVNILLFMG	YMTKDNLAIV	360
361	TQWCEGSSLY	KHLHVQETKF	QMFQLIDIAR	QTAQGMDYLH	AKNIIHRDMK	SNNIFLHEGL	420
421	TVKIGDFGLA	TVKSRWGSQ	QVEQPTGSVL	WMAPEVIRMQ	DNNPFSFQSD	VYSYGIVLYE	480
481	LMTGELPYSH	INNDRQIIFM	VGRGYASPD	SKLYKNCPKA	MKRLVADCVK	KVKEERPLFP	540
541	QILSSIQLQ	HSLPKINRSA	SEPSLHRAAH	TEDINACTLT	TSPRLPVF		600

1-218: GST    **Red**: HIS6-tag    **Green**: 3C cleavage site    **blue**: RAF1 fragment    **DD**: YDYD mutation

RAF1 wt <sup>1</sup> Amino Acid Sequence							
1	MEHIQGAWKT	ISNGFGFKDA	VFDGSSCISP	TIVQQFGYQR	RASDDGKLT	PSKTSNTIRV	60
61	FLPNKQRTVV	NVRNGMSLHD	CLMKALKVRG	LQPECCAVER	LLHEHKGKKA	RLDWNTDAAS	120
121	LIGEELQVDF	LDHVPLTTHN	FARKTFLKLA	FCDICQKFL	NGFRCQTCGY	KFHEHCSTKV	180
181	PTMVDWSNI	RQLLLFPNST	IGDSGVPALP	SLTMRRMRES	VSRMPVSSQH	RYSTPHAFTF	240
241	NTSSPSSEGS	LSQRQRSTST	PNVHVMVSTL	PVDSRMIEDA	IRSHSESASP	SALSSSPNNL	300
301	SPTGWSQPKT	PVPAQRERAP	VSGTQEKNKI	RPRGQRDSSY	YWEIEASEVM	LSTRIGSGSF	360
361	GTVYKGGKWHG	DVAVKILKVV	DPTPEQFQAF	RNEVAVLRKT	RHVNILLFMG	YMTKDNLAIV	420
421	TQWCEGSSLY	KHLHVQETKF	QMFQLIDIAR	QTAQGMDYLH	AKNIIHRDMK	SNNIFLHEGL	480
481	TVKIGDFGLA	TVKSRWGSQ	QVEQPTGSVL	WMAPEVIRMQ	DNNPFSFQSD	VYSYGIVLYE	540
541	LMTGELPYSH	INNDRQIIFM	VGRGYASPD	SKLYKNCPKA	MKRLVADCVK	KVKEERPLFP	600
600	QILSSIQLQ	HSLPKINRSA	SEPSLHRAAH	TEDINACTLT	TSPRLPVF		660

**blue**: RAF1 sequence expressed in recombinant protein    **Red**: variant in recombinant protein

<sup>1</sup>[NCBI/Protein](https://www.ncbi.nlm.nih.gov/protein/NP_002871.1) accession number NP\_002871.1

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