

ProQinase™ RAF1 Y340D/Y341D (untagged)

Raf-1 proto-oncogene, serine/threonine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: RAF1

Synonyms: cRAF, CRAF, NS5

Product No.: 0792-0000-2

Lot: 002

Description: Human RAF1 C-terminal fragment, amino acids S₃₀₆-F₆₄₈ (as in [NCBI/Protein](#) entry NP_002871.1), Y₃₄₀D, Y₃₄₁D mutant, untagged, expressed in Sf9 insect cells

Product identity: RAF1 Y340D/Y341D Lot 002 was confirmed as RAF1 by mass spectroscopy LC-ESI-MS/MS

Theoretical MW_{Fusion Protein}: 38,472 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was activated by introducing the Y₃₄₀D, Y₃₄₁D point mutations

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

Storage temperature: -80°C

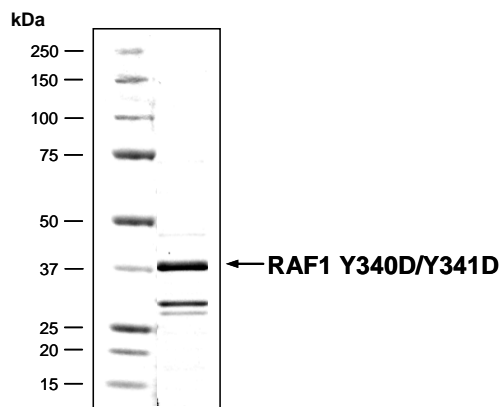
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.334 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

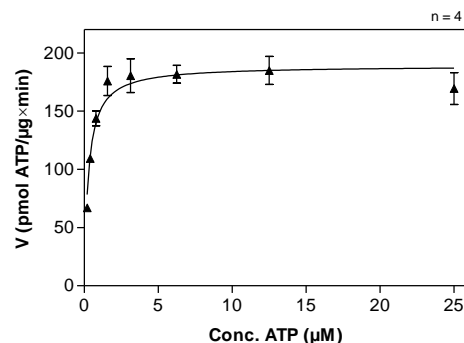
Specific kinase activity (P_i transfer): 189 pmol/µg × min
ATP-K_M: 0.28 µM

RAF1 YDYD Lot 002: Coomassie stain



2.0 µg RAF1 Y340D/Y341D

RAF1 YDYD Lot 002: 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 MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg / ml PEG_{20,000}
 - ATP (variable)
 - Substrate: MEK1-K₉₇M, 40 µg/ml
 - Kinase: 400 ng/ml
- Filter binding assay
MSFC membrane (Millipore)

Additional assay technology:

RAF1 YDYD Lot 002 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

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RAF1 Y340D/Y341D Recombinant Protein Amino Acid Sequence							
1	GPLAMGSQPK	TPVPAQRERA	PVSGTQEKNK	IRPRGQRDSS	DDWEIEASEV	MLSTRIGSGS	60
61	FGTVYKQKWH	GDVAVKILKV	VDPTPEQFQA	FRNEVAVLRK	TRHVNILLFM	GYMTKDNLA	120
121	VTQWCEGSSL	YKHLHVQETK	FQMFQLIDIA	RQTAQGMDYL	HAKNIIHRDM	KSNNIFLHEG	180
181	LTVKIGDFGL	ATVKSRSWGS	QQVEQPTGSV	LWMAPEVIRM	QDNNPFSFQS	DVYSYGIVLY	240
241	ELMTGELPYS	HINNRDQIIF	MVGRGYASPD	LSKLYKNCPK	AMKRLVADCV	KKVKEERPLF	300
301	PQILSSIPELL	QHSLPKINRS	ASEPSLHRAA	HTEDINACTL	TSPRLPVF		360

1-6: legacy of tag-cleavage **blue**: RAF1 fragment **boxed**: Y340D/Y341D mutation

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

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

¹[NCBI/Protein](#) accession number NP_002871.1