Certificate of Analysis

REACTION BIOLOGY

ProQinase™ ARAF YDYD

A-Raf proto-oncogene, serine/threonine kinase

Recombinant Human Active Protein Kinase

HGNC Symbol: ARAF

Synonyms: ARAF1

Product No.: 1768-0000-1

Lot: 004

Description: Human ARAF, C-terminal fragment, amino acids K_{282}-P_{606} (as in NCBI/Protein entry NP_001645.1), point mutations $Y_{301}D/Y_{302}D$, N-terminal GST-HIS_6 fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

Product identity: ARAF YDYD LOT004, has been verified by mass spectrometry LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 65578 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

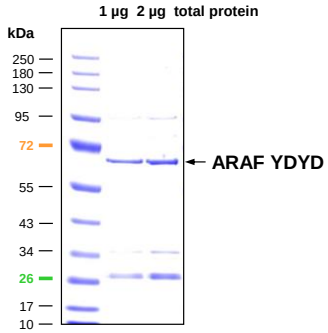
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

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!

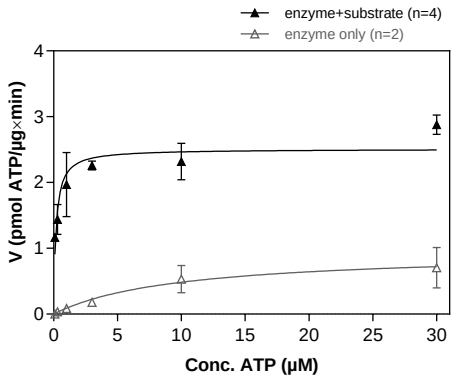
Protein concentration: 0.09 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:
Specific kinase activity (P_i transfer): 2.5 pmol/µg*min
ATP-K_M: 0.2 µM

ARAF YDYD LOT004
Coomassie stain



ARAF YDYD LOT004
Determination of V_{max} and K_M value for ATP



- 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: 4 µg/ml

Assay technology:
Radiometric filter binding assay
MSFC membrane (96 well plate, Millipore)

Recombinant Proteins

Sequence information

GST-ARAF 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 KMFKDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK 180
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RDSLEVLFQG 240
241  PLAMGARGRK SLADDKKKVK NLGYRDSGDD WEVPPSEVQL LKRIGTGSFG TVFRGRWHGD 300
301  VAVKVLKVSQ PTAEQAQAFK NEMQVLRKTR HVNILLFMGF MTRPGFAIIT QWCEGSSLYH 360
361  HLHVADTRFD MVQLIDVARQ TAQGMDYLHA KNIIHRDLKS NNIFLHEGLT VKIGDFGLAT 420
421  VKTRWSGAQP LEQPSGSVLW MAAEVIRMQD PNPYSFQSDV YAYGVVLYEL MTGSLPYSHI 480
481  GCRDQIIFMV GRGYLSPDLS KISSNCPKAM RRLLSDCLKF QREERPLFPQ ILATIELLQR 540
541  SLPKIERSAS EPSLHRTQAD ELPACLLSAA RLVP                              600
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1-218: GST **Red**: HIS6-tag **Green**: 3C cleavage site **blue**: ARAF fragment **boxed**: $Y_{301}DY_{302}D$ double mutation

ARAF wt[1] Amino Acid Sequence

```
  1  MEPPRGPPAN GAEPSRAVGT VKVYLPNKQR TVVTVRDGMS VYDSLDKALK VRGLNQDCCV  60
 61  VYRLIKGRKT VTAWDTAIAP LDGEELIVEV LEDVPLTMHN FVRKTFFSLA FCDFCLKFLF 120
121  HGFRCQTCGY KFHQHCSSKV PTVCVDMSTN RQQFYHSVQD LSGGSRQHEA PSNRPLNELL 180
181  TPQGPSPRTQ HCDPEHFPFP APANAPLQRI RSTSTPNVHM VSTTAPMDSN LIQLTGQSFS 240
241  TDAAGSRGGS DGTPRGSPSP ASVSSGRKSP HSKSPAEQRE RKSLADDKKK VKNLGYRDSG 300
301  YYWEVPPSEV QLLKRIGTGS FGTVFRGRWH GDVAVKVLKV SQPTAEQAQA FKNEMQVLRK 360
361  TRHVNILLFM GFMTRPGFAI ITQWCEGSSL YHHLHVADTR FDMVQLIDVA RQTAQGMDYL 420
421  HAKNIIHRDL KSNNIFLHEG LTVKIGDFGL ATVKTRWSGA QPLEQPSGSV LWMAAEVIRM 480
481  QDPNPYSFQS DVYAYGVVLY ELMTGSLPYS HIGCRDQIIF MVGRGYLSPD LSKISSNCPK 540
541  AMRRLLSDCL KFQREERPLF PQILATIELL QRSLPKIERS ASEPSLHRTQ ADELPACLLS 600
601  AARLVP                                                            660
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blue: ARAF sequence expressed in recombinant protein **Red**: variant in recombinant protein

[1]NCBI/Protein accession number NP_001645.1

Recombinant Proteins