

## 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: 001

**Description:** Human ARAF, C-terminal fragment, amino acids K<sub>282</sub>-P<sub>606</sub> (as in [NCBI/Protein](#) entry NP\_001645.1), point mutations Y<sub>301</sub>D/Y<sub>302</sub>D, N-terminal GST-HIS<sub>6</sub> fusion protein with a 3C cleavage site, expressed in Sf9 insect cells

**Product identity:** ARAF YDYD Lot 001, was confirmed as ARAF by mass spectroscopy LC-ESI-MS/MS

**Theoretical MW<sub>Fusion Protein</sub>:** 65,578 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:** 0.474 µg/µl

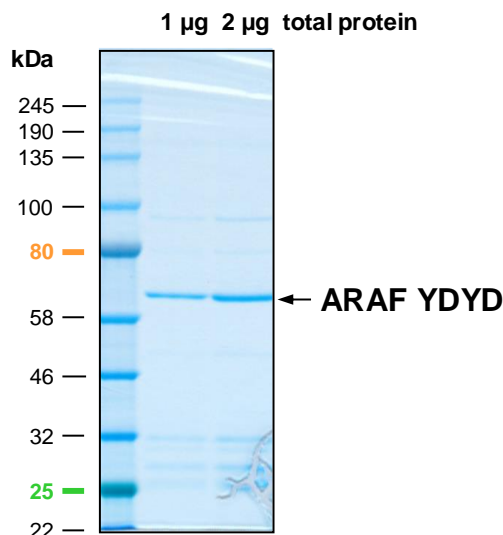
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

### Biochemical Parameters:

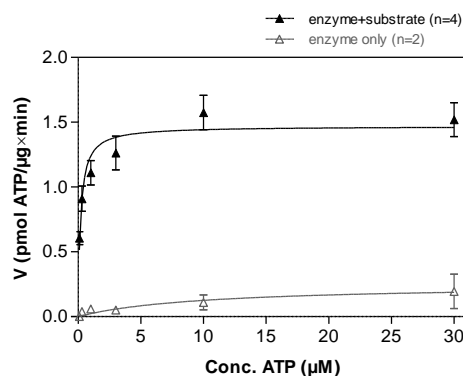
Specific kinase activity (P<sub>i</sub> transfer): 1.5 pmol/µg × min

ATP-K<sub>M</sub>: 0.2 µM

### ARAF YDYD Lot 001: Coomassie stain



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



- 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: 4 µg/ml
- Filter binding assay (radiometric)  
MSFC membrane (Millipore)

## ARAF YDYD

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### GST-ARAF YDYD Recombinant Fusion Protein Amino Acid Sequence

1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFKDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RDSLEVLFGQ	240
241	PLAMGARGRK	SLADDDKKVK	NLGYRDSGDE	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	RRLSDCLKF	QREERPLFPQ	ILATIELLQR	540
541	SLPKIERSAS	EPSLHRTQAD	ELPACLLSAA	RLVP			600

1-218: GST Red: HIS6-tag Green: 3C cleavage site blue: ARAF fragment boxed: Y<sub>301</sub>DY<sub>302</sub>D double mutation

### ARAF wt<sup>1</sup> Amino Acid Sequence

1	MEPPRGPPAN	GAEPSRAVGT	VKVYLPNKQR	TVVTVRDGMS	VYDSLKALK	VRGLNQDCCV	60
61	VYRLIKGRKT	VTAWDTAIAP	LDGEELIVEV	LEDVPLTMHN	FVRKTFSLA	FCDFCLKFLF	120
121	HGFRCQTCGY	KFHQHCSSKV	PTVCVDMSTN	RQQFYHSVQD	LSGGSRQHEA	PSNRPLNELL	180
181	TPQGSPRTQ	HCDPEHFPPF	APANAPLQRI	RSTSTPNVHM	VSTTAPMDSN	LIQLTGQSFS	240
241	TDAAGSRGGS	DGTPRGSPSP	ASVSSGRKSP	HSKSPAEQRE	RKSLADDDKKK	VKNLGYRDSG	300
301	YYWEVPPSEV	QLLKRIQTGS	FGTVFRGRWH	GDVAVKVLKV	SQPTAEQAQA	FKNEMQVLRK	360
361	TRHVNILLFM	GFMRPGFAI	ITQWCEGSSL	YHHLHVADTR	FDMVQLIDVA	RQTAQGMDYL	420
421	HAKNIIHRDL	KSNIFLHEG	LTVKIGDFGL	ATVKTRWSGA	QPLEQPSGSV	LWMAAEVIRM	480
481	QDPNPYSFQS	DVYAYGVVLY	ELMTGSLPVS	HIGCRDQIIF	MVGRGYLSPD	LSKISSNCPK	540
541	AMRRLSDCL	KFQREERPLF	PQILATIELL	QRSPLKIER	ASEPSLHRTQ	ADELPACLLS	600
601	AARLVP						660

blue: ARAF sequence expressed in recombinant protein Red: variant in recombinant protein

<sup>1</sup>[NCBI/Protein](#) accession number NP\_001645.1