

Certificate of Analysis

ACV-R1

Homo sapiens activin A receptor, type I

Recombinant Human Active Protein Kinase

HGNC Symbol: ACVR1

Synonyms: ALK2, SKR1, ACTRI, ACVRLK2

Product No.: 0372-0000-1

Lot: 001

Description: Human ACV-R1, C-terminal fragment, amino acids A₁₄₅-C₅₀₉ (as in NCBI/Protein entry NP_001096.1), N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: ACV-R1 Lot 001, was confirmed as ACV-R1 by specific Western Blotting using anti ACV-R1 antibody

Theoretical MW_{Fusion Protein}: 71,534 Da

Expression: Baculovirus infected Sf9 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, 4 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: 0.391 µg/µl

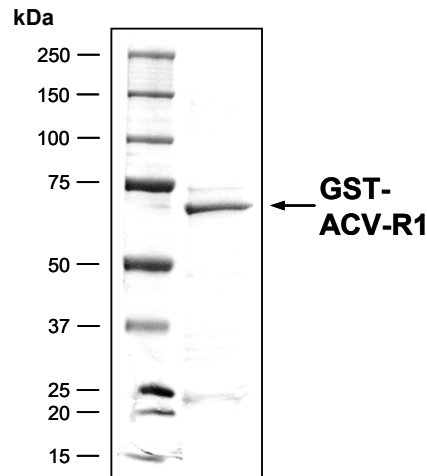
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

Specific kinase activity (P_i transfer): 92 pmol/µg×min

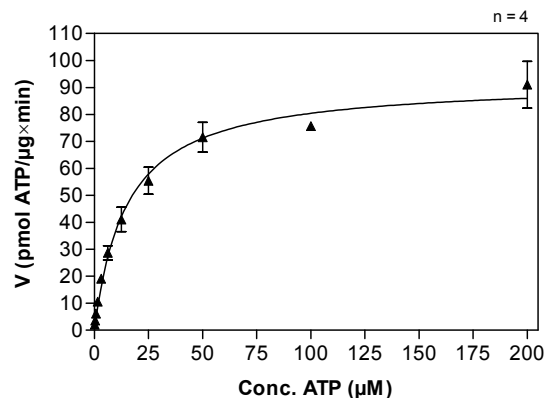
ATP-K_M: 15 µM

ACV-R1 Lot 001: Coomassie stain



2.0 µg GST-ACV-R1

ACV-R1 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 MgCl₂
 - 3 mM MnCl₂
 - 3 µM Na-orthovanadate
 - 1.2 mM DTT
 - 50 µg / ml PEG_{20,000}
 - ATP (variable)
 - Substrate: Casein, 200 µg/ml
 - ACV-R1: 4.0 µg/ml
- Filter binding assay
 - MSFC membrane (Millipore)

Additional assay technology: ACV-R1 Lot 001

was also successfully tested by ProQinase for the use with the ADP-Glo™ Kinase assay from Promega



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ACV-R1 Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLP EML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAI PQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHGG	RRRASVAAGI	240
241	LVPRG SGLD	GIYARGIQAS	MALRKFKRRN	QERLNPRDVE	YGTIEGLITT	NVGDSTLADL	300
301	LDHSCTSGSG	SGLPFLVQRT	VARQITLLEC	VGKGRYGEVW	RGSWQGENVA	VKIFSSRDEK	360
361	SWFRET ELYN	TVMLRHENIL	GFIASDMTSR	HSSTQLWLIT	HYHEMGS LYD	YLQLTTLDTV	420
421	SCLRIVLSIA	SGLAHLHIEI	FGTQ GKPAIA	HRDLKSKNIL	VKKNQCCIA	DLGLAVMHSQ	480
481	STNQLDVGN	PRVGT KRYMA	PEVLDETIQV	DCFDSYKRVD	IWAFGLVLWE	VARRMVSNGI	540
541	VEDYKPPFYD	VVPNDPSFED	MRKVVCVDQQ	RPNIPNRWFS	DPTLTSLAKL	MKECWYQNPS	600
601	ARLTALRIKK	TLTKIDNSLD	KLKTDC				660

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: ACV-R1 fragment

ACV-R1 wt ¹ Amino Acid Sequence							
1	MVDGVMILPV	LIMIALPSPS	MEDEKPKVNP	KLYMCVCEGL	SCGNEDHCEG	QQCFSSLSIN	60
61	DGFH VYQKGC	FQVYEQ GKMT	CKTPPSPGQA	VECCQGDWCN	RNITAQLPTK	GKSFPGTQNF	120
121	HLEVGLI ILS	VVFAVCLLAC	LLGV ALRKFK	RRNQERLNPR	DVEYGTIEGL	ITTNVGDSTL	180
181	ADLLDH SCTS	GSGLPFLV	QRTVARQITL	LECVGKGRYG	EVWRG SWQGE	NVAVKIFSSR	240
241	DEKSWFRETE	LYNTV MLRHE	NILGFIASDM	TSRHSSTQLW	LITHYHEMGS	LYDYLQLTTL	300
301	DTV SCLRIVL	SIASGLAHLH	IEIFGTQGKP	AIAHRDLKSK	NILVKKNQOC	CIADLGLAVM	360
361	HSQSTNQLDV	GNNPRVGT KR	YMAPEVLDET	IQVDCFDSYK	RVDI WAFGLV	LWEVARRMVS	420
421	NGIVEDYKPP	FYDVVPNDPS	FEDMRKVVCV	DQQRPNIPNR	WFS DPTLTSL	AKLMKECWYQ	480
481	NPSARLTALR	IKKTLTKIDN	SLDKLKTDC				540

blue: ACV-R1 sequence expressed in fusionprotein

¹NCBI/Protein accession number NP_001096.1