

HRI

eukaryotic translation initiation factor 2 alpha kinase 1

Recombinant Human Active Protein Kinase

HGNC Symbol: EIF2AK1

Synonyms: HCR, PRO1362

Product No.: 0444-0000-1

Lot: 001

Description: Human HRI, full length, amino acids M1-G₆₃₀ (as in [NCBI/Protein](#) entry NP_055228.2), ΔA₂₄₄, N-terminal GST-HIS₆ fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

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

Theoretical MW_{Fusion Protein}: 100,546 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, 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: 0.03 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

Biochemical Parameters:

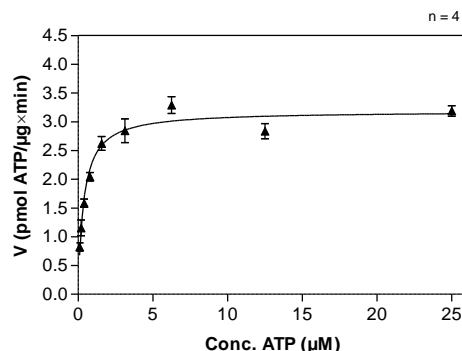
Specific kinase activity (P_i transfer): 3.2 pmol/µg × min

ATP-K_M: 0.36 µM

HRI Lot 001: Coomassie stain



HRI 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 20 µg/ml
 - Kinase: 4 µg/ml
- Filter binding assay
- MSFC membrane (Millipore)

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GST-HRI Recombinant Fusion Protein Amino Acid Sequence							
1	MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID	60
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV	120
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK	180
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	PMGHHHHHG	RRRASVAAGI	240
241	LVPRGSPGLD	GIYARAMQGG	NSGVRKREEE	GDGAGAVAAP	PAIDFPAEGP	DPEYDESDVP	300
301	AEIQVLKEPL	QQPTFFFAVA	NQLLLVSLLE	HLSHVHEPNP	LRSRQVFKLL	CQTFIKMGLL	360
361	SSFTCSDEFS	SLRLHNNRAI	THLMRSAKER	VRQDPCEDIS	RIQKIRSREV	ALEAQTSTRYL	420
421	NEFEELAILG	KGGYGRVYKV	RNKLDGQYYA	IKKILIKGAT	KTVCMKVLR	VKVLAGLQHP	480
481	NIVGYHTAWI	EHVHVIQPRD	RAAIELPSLE	VLSDQEEDRE	QCGVKNDSS	SSSIIFAEPT	540
541	PEKEKRFGES	DTENQNNKSV	KYTTNLVIRE	SGELESTLEL	QENGLAGLSA	SSIVEQQLPL	600
601	RNSHLEESF	TSTESSEEN	VNFLGQTEAQ	YHMLHIQMQ	LCELSLWDWI	VERNKRGREY	660
661	VDESACPYVM	ANWATKIFQE	LVEGVFYIHN	MGIVHRDLKP	RNIFLHGPDQ	QVKIGRGLA	720
721	CTDILQKNTD	WTNRNGKRTF	THTSRVGTCL	YASPEQLEGS	EYDAKSDMYS	LGVLLELFQ	780
781	PFGTEMERAE	VLTGLRTGQL	PESLRKRCVP	QAKYIQHLTR	RNSSQRPSAI	QLLQSELFQN	840
841	SGNVNLTLOM	KIIEQEKEIA	ELKKQLNLLS	QDKGVRDDGK	DGGVG		900

1-218: GST Red: HIS6-tag Pink: Thrombin cleavage site blue: HRI

HRI wt ¹ Amino Acid Sequence							
1	MQGGNSGVRK	REEEGDGAGA	VAAPPAIDFP	AEGPDPEYDE	SDVPAEIQVL	KEPLQOPTFP	60
61	FAVANQLLLV	SLLEHLSHVH	EPNPLRSRQV	FKLLCQTFIK	MGLLSSFTCS	DEFSSRLRHH	120
121	NRAITHLMRS	AKERVQRQPC	EDISRIQKIR	SREVALEAQT	SRYLNEFEEL	AILGKGGYGR	180
181	VYKVRNKLDG	QYYAIIKILI	KGATKTVCMK	VLREVKVLAG	LQHPNIVGYH	TAWIEHVHVI	240
241	QPRADRAAIE	LPSLEVLSQ	EEDREQCGVK	NDESSSSII	FAEPTPEKEK	RFGESDTENQ	300
301	NNKSVKYTTN	LVIRESGELE	STLELQENGL	AGLSASSIVE	QQLPLRRNSH	LEESFTSTEE	360
361	SSEENVNVLG	QTEAQYHML	HIQMQLCELS	LWDWIVERNK	RGREYVDESA	CPYVMANVAT	420
421	KIFQELVEGV	FYIHNMGIVH	RDLKPRNIFL	HGPDQQVKIG	DFGLACTDIL	QKNTDWTNRN	480
481	GKRTPTHTSR	VGTCLYASPE	QLEGSEYDAK	SDMYSLGVVL	LELFQPFQTE	MERAEVLTGL	540
541	RTGQLPESLR	KRCVPQAKYI	QHLTRRNSSQ	RPSAIQLLOS	ELFQNSGNVN	LTLQMKIIEQ	600
601	EKEIAELKKQ	LNLLSQDKGV	RDDGKDDGGV				660

blue: HRI sequence expressed in recombinant protein Red: variant (missing) in recombinant protein

¹NCBI/Protein accession number NP_055228.2