

ProQinase™ 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)

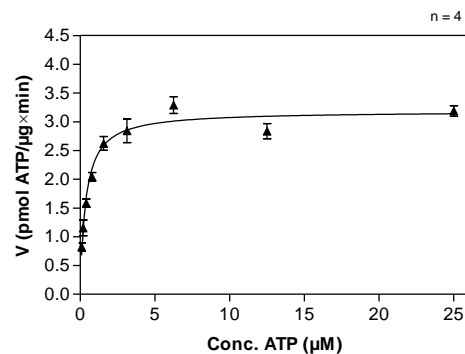
Biochemical Parameters:

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	HLIERDEGDK	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	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	ALEAQT SRYL	420
421	NEFEELAILG	KGGYGRVYKV	RNKLDGQYYA	IKKILIKGAT	KTVCMKV LRE	VKVLAGLQHP	480
481	NIVGYHTAWI	EHVHVIQPRD	RAAIELPSLE	VLSDQEEDRE	QCGVKND ESS	SSSIIFAEPT	540
541	PEKEKRFGES	DTENQNNKSV	KYTTNLVIRE	SGELESTLEL	QENGLAGLSA	SSIVEQQ LPL	600
601	RRNSHLEESF	TSTEESS EEN	VNFLGQTEAQ	YHMLLHIQMQ	LCELSLWDWI	VERNKRGREY	660
661	VDESACPYVM	ANVATKIFQE	LVEGVFYIHN	MGIVHRDLKP	RNIFLHGPDQ	QVKIGDFGLA	720
721	CTDILQKNTD	WTNRNGK RTP	THTSRVGTCL	YASPEQLEGS	EYDAKSDMYS	LGVLLELFQ	780
781	PFGTEMERAE	VLTGLRTGQL	PESLRKRCPV	QAKYIQHLTR	RNSSQRPSAI	QLLQSELFQN	840
841	SGNVNLT LQM	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	SLLEHL SHVH	EPNPLRSRQV	FKLLCQTFIK	MGLLSSFTCS	DEFSSRLRHH	120
121	NRAITHLMRS	AKERV RQDPC	EDISRIQKIR	SREVALEAQT	SRYLNEFEEL	AILGKGGYGR	180
181	VYKVRNKLDG	QYYA IKKILI	KGATKTVCMK	VLREVKVLAG	LQHPNIVGYH	TAWIEHVHVI	240
241	QPRADRAAIE	LPSLEVLSDQ	EEDREQCGVK	NDESSSSSII	FAEPTPEKEK	RFGESDTENQ	300
301	NNKSVKYTTN	LVIRESGELE	STLELQENGL	AGLSASSIVE	QQLPLRRNSH	LEESFTSTEE	360
361	SSEENVNFLG	QTEAQYHML	HIQMQLCELS	LWDWIVERNK	RGREYVDESA	CPYVMANVAT	420
421	KIFQELVEGV	FYIHNMGIVH	RDLKPRNIFL	HGPDQQVKIG	DFGLACTDIL	QKNTDWTNRN	480
481	GKRTPTHTSR	VGTCLYASPE	QLEGSEYDAK	SDMYSLGVVL	LELFQPFQTE	MERA EVL TGL	540
541	RTGQLPESLR	KRCPVQAKYI	QHLTRRNSSQ	RPSAIQLLQS	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