

Certificate of Analysis

PKR, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-955, 14-955-K, 14-955M

Parent Lot # D14NP013N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run

Product Description: N-terminal GST-tagged and C-terminal 10His tagged, recombinant, human PKR amino acids 252-end, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 85% by SDS-PAGE and Coomassie blue staining. MW = 64kDa.

Specific Activity (Parent lot# D14NP013N):

1189U/mg, where one unit of PKR, active activity is defined as 1nmol phosphate incorporated into 30 μ M (RSRSRSRSRSRSRSR) per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 0.09mg/ml of enzyme in 40mM Tris/HCl pH7.5, 120mM NaCl, 240mM imidazole, 0.08mM EGTA, 20% (v/v) glycerol, 0.8mM benzamidine, 0.16mM PMSF, 0.08% 2-mercaptoethanol. Frozen solution.

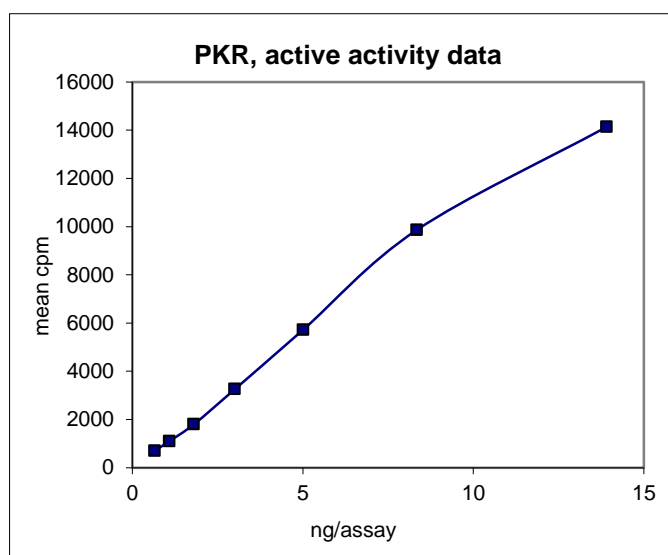
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

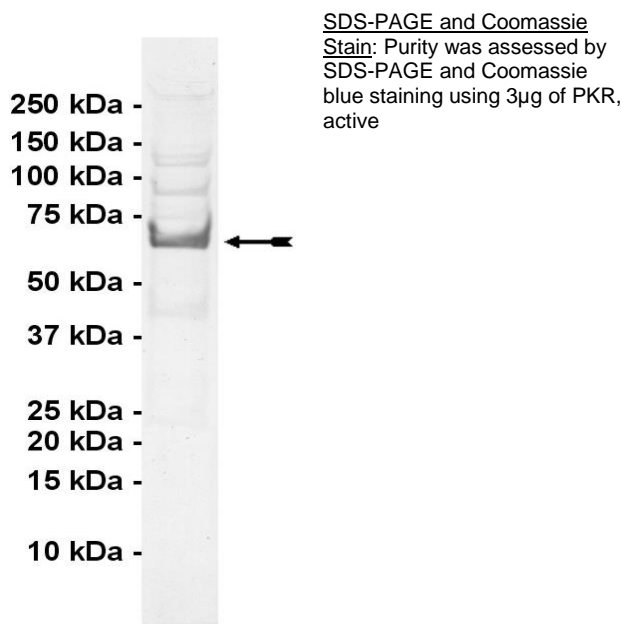
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 0.7–13.9ng of this lot of enzyme phosphorylated 30 μ M (RSRSRSRSRSRSRSR) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as PKR with the translated sequence listed on page three



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **(RSRSRSRSRSRSRSR):** Use at a final assay concentration of 30 μ M. Prepare a 3mM stock and add 0.25 μ l of stock per assay point.
3. **PKR, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 0.7–13.9ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x MgAc/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer per assay to wells.
2. Add 0.25 μ l of **(RSRSRSRSRSRSRSR)**.
3. Add **2.5 μ l (0.7–13.9ng) PKR, active**.
4. Add 7.25 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l of 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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PKR Sequence Information

Protein	human PKR
Tags	N-terminal GST and C-terminal 10His
Native sequence	M239 of the recombinant protein is equivalent to M252 of human PKR
Accession number	GenBank NM_002759.1

Recombinant PKR amino acid sequence:

1	MSPILGYWKI	KGLVQPTRLL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID
61	GDVKLTQSMA	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV
121	DFLSKLPPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK
181	KRIEAIPOID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSHNQT	SLYKKAGTMK
241	ETKYTVDKRF	GMDFKEIELI	GSGGFGQVFK	AKHRIDGKTY	VIKRVKYNNE	KAEREVKALA
301	KLDHVNIIVHY	NGCWDGFDYD	PETSDDSLES	SDYDPENSKN	SSRSKTKCLF	IQMEFCDKGT
361	LEQWIEKRRG	EKLDKVLALE	LFEQITKGVD	YIHSKKLIHR	DLKPSNIFLV	DTKQVKIGDF
421	GLVTSLKNDG	KRTRSKGTLR	YMSPEQISSQ	DYGKEVDLYA	LGLILAEELLH	VCDTAFETSK
481	FFTDLRDGI I	SDIFDKKEKT	LLQKLLSKKP	EDRPNTSEIL	RTLTVWKKSP	EKNERHTCGP
541	GPGHHHHHHH	HHH				

Recombinant PKR nucleotide sequence:

1	atgtccccta	tactaggtta	ttggaaaatt	aagggccttg	tgcaaccac	tcgacttctt
61	ttggaatata	ttgaagaaaa	atatgaagag	catttgtatg	agcgcgatga	aggtgataaa
121	tggcgaaaca	aaaagtttga	attgggtttg	gagtttccca	atcttcctta	ttatattgat
181	ggtgatgtta	aattaacaca	gtctatggcc	atcatacggt	atatagctga	caagcacaac
241	atgttgggtg	gttgtccaaa	agagcgtgca	gagatttcaa	tgcttgaagg	agcgggtttg
301	gatattagat	acggtgtttc	gagaattgca	tatagtaaag	actttgaaac	tctcaaagtt
361	gattttctta	gcaagctacc	tgaaatgctg	aaaatgttcg	aagatcgttt	atgtcataaa
421	acataattaa	atggtgatca	tgtaacccat	cctgacttca	tgttgataga	cgctcttgat
481	gttgttttat	acatggaccc	aatgtgcctg	gatgcgttcc	caaaattagt	ttgttttaaa
541	aaacgtattg	aagctatccc	acaaattgat	aagtacttga	aatccagcaa	gtatatagca
601	tggcctttgc	agggctggca	agccacgttt	ggtggtggcg	accatcctcc	aaaatcggat
661	ctggttccgc	gtggatccca	caaccagacg	tcgctataca	aaaaagccgg	cacgatgaaa
721	gaaacaaagt	atactgtgga	caagaggttt	ggcatggatt	ttaaagaaat	agaattaatt
781	ggctcagggtg	gattttggcca	agttttcaaa	gcaaaacaca	gaattgacgg	aaagacttac
841	gttattaaac	gtgttaaata	taataacgag	aaggcggagc	gtgaagtaaa	agcattggca
901	aaacttgatc	atgtaaatat	tgttcactac	aatggctggt	gggatggatt	tgattatgat
961	cctgagacca	gtgatgattc	tcttgagagc	agtgattatg	atcctgagaa	cagcaaaaaat
1021	agttcaaggt	caaagactaa	gtgccttttc	atccaaatgg	aattctgtga	taaagggacc
1081	ttggaacaat	ggattgaaaa	aagaagaggc	gagaaactag	acaaagtttt	ggctttggaa
1141	ctctttgaac	aaataacaaa	aggggtggat	tatatacatt	caaaaaaatt	aattcataga
1201	gatcttaagc	caagtaatat	attcttagta	gatacaaaac	aagtaaagat	tggagacttt
1261	ggacttgtaa	catctctgaa	aaatgatgga	aagcgaacaa	ggagtaaggg	aactttgcga
1321	tacatgagcc	cagaacagat	ttcttcgcaa	gactatggaa	aggaagtgga	cctctacgct
1381	ttggggctaa	ttcttgctga	acttcttcac	gtatgtgaca	ctgcttttga	aacatcaaag
1441	tttttcacag	acctacggga	tggcatcatc	tcagatatac	ttgataaaaa	agaaaaaact
1501	cttctacaga	aattactctc	aaagaaacct	gaggatcgac	ctaacacatc	tgaataacta
1561	aggaccttga	ctgtgtggaa	gaaaagccca	gagaaaaatg	aacgacacac	atgtggcccc
1621	ggccctggcc	atcaccatca	ccatcaccat	caccatcact	aa	

Reviewed and approved by site quality representative.

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