

Certificate of Analysis

Plk1, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-777, 14-777-K, 14-777M

Parent Lot # D7SN005U

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 6His tagged, recombinant, human Plk1, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺-NTA agarose. Purity 91.7% by SDS-PAGE and Coomassie blue staining. MW = 69.3kDa.

Specific Activity (Parent lot# D7SN005U): 60U/mg, where one unit of Plk1, active activity is defined as 1nmol phosphate incorporated into 1.25mg/ml casein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.301mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

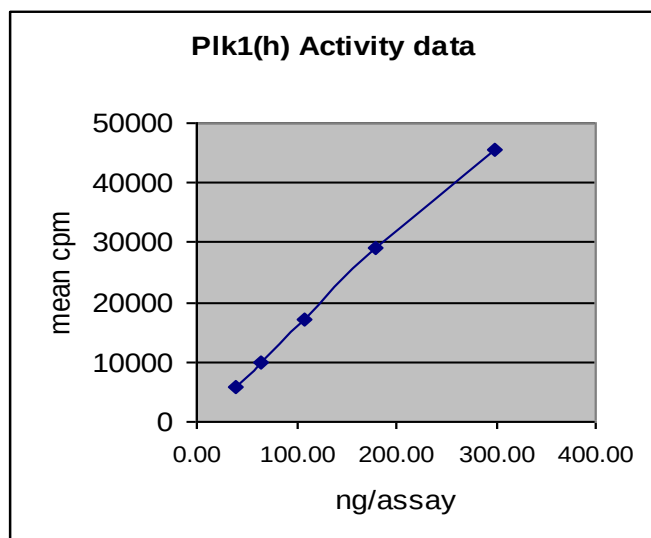
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

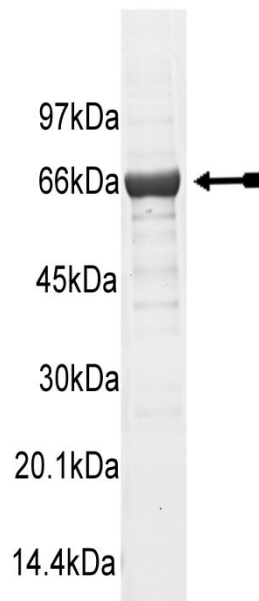
Quality Control Testing

Kinase Assay: 39–299ng of this lot of enzyme phosphorylated 1.25mg/ml casein 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 Plk1 with the translated sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active Plk1.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **DTT:** Use at a final assay concentration of 20mM. Prepare a 1M stock. Add 0.5µl of stock per assay point.
3. **Casein:** Use at a final assay concentration of 1.25mg/ml. Prepare a 12.5mg/ml stock. Add 2.5µl of stock per assay point.
4. **Plk1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 39–299ng per assay point.
5. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[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 5µl of 5 x reaction buffer per assay to wells.
2. Add 0.5µl of DTT.
3. Add 2.5µl of **casein**.
4. Add **2.5µl (39–299ng) Plk1, active**.
5. Add 4.5µl of dH₂O.
6. Add 10µl of diluted [γ -³³P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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Plk1 Sequence Information

<u>Protein</u>	human Plk1
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M8 of the recombinant protein is equivalent to M1 of human Plk1
<u>Accession number</u>	GenBank NM_005030

Recombinant Plk1 amino acid sequence:

1	MHHHHHHMSA	AVTAGKLARA	PADPGKAGVP	GVAAPGAPAA	APPAKEIPEV	LVDPRSRRRY
61	VRGRFLGKGG	FAKCFEISDA	DTKEVFAGKI	VPKSLLLKPH	QREKMSMEIS	IHRSLAHQHV
121	VGFGHGFEDN	DFVFVVLELC	RRRSLLLEHK	RRKALTEPEA	RYYLRLQIVLG	CQYLHRNRVI
181	HRDLKLGRLF	LNEDLEVKIG	DFGLATKVEY	DGERKKTLCG	TPNYIAPEVL	SKKGHSFEVD
241	VWSIGCIMYT	LLVGKPPFET	SCLKETYLRI	KKNEYSIPKH	INPVAASLIQ	KMLQTDPTAR
301	PTINELLNDE	FFTSGYIPAR	LPITCLTIPP	RFSIAPSSLD	PSNRKPLTVL	NKGLNPLPE
361	RPREKEEPV	RETGEVVDCH	LSDMLQQLHS	VNASKPSERG	LVRQEEAEDP	ACIPIFWVSK
421	WVDYSDKYGL	GYQLCDNSVG	VLFDNDSTRLI	LYNDGDSLQY	IERDGTESYL	TVSSHPNSLM
481	KKITLLKYFR	NYMSEHLLKA	GANITPREGD	ELARLPYLRT	WFRTRSAIL	HLSNGSVQIN
541	FFQDHTKLIL	CPLMAAVTYI	DEKRDFRTYR	LSLLEEYGCC	KELASRLRYA	RTMVDKLLSS
601	RSASNRLKAS					

Recombinant Plk1 nucleotide sequence:

1	atgcaccatc	accatcacca	tatgagtgc	gcagtgcactg	caggggaagct	ggcacgggca
61	ccggccgacc	ctgggaaagc	cggggtcccc	ggagttgcag	ctcccgagac	tccggcggcg
121	gctccaccgg	cgaaagagat	cccggaggtc	ctagtggacc	cacgcagccg	gcgccgctat
181	gtgcggggcc	gctttttggg	caagggcggc	tttgccaagt	gcttcgagat	ctcggacgcg
241	gacaccaagg	aggtgttcgc	gggcaagatt	gtgcctaagt	ctctgctgct	caagccgcac
301	cagagggaga	agatgtccat	ggaaatatcc	attcaccgca	gcctcgccca	ccagcacgtc
361	gtaggattcc	acggcttttt	cgaggacaac	gacttcgtgt	tcgtggtggt	ggagctctgc
421	cgccggaggt	ctctcctgga	gctgcacaag	aggaggaaaag	ccctgactga	gcctgaggcc
481	cgatactacc	tacggcaa	tgtgcttggc	tgccagtacc	tgcaccgaaa	ccgagttatt
541	catcgagacc	tcaagctggg	caaccttttc	ctgaatgaag	atctggaggt	gaaaataggg
601	gatttttgac	tggcaaccaa	agtcgaatat	gacggggaga	ggaagaagac	cctgtgtggg
661	actcctaatt	acatagctcc	cgaggtgctg	agcaagaaaag	ggcacagttt	cgaggtggat
721	gtgtggtcca	ttgggtgtat	catgtatacc	ttgttagtgg	gcaaaccacc	ttttgagact
781	tcttgcctaa	aagagacctt	cctccggatc	aagaagaatg	aatacagtat	tcccaagcac
841	atcaaccctg	tggccgcctc	cctcatccag	aagatgcttc	agacagatcc	cactgcccgc
901	ccaaccatta	acgagctgct	taatgacgag	ttctttactt	ctggctatat	ccctgccgtg
961	ctccccatca	cctgcctgac	cattccacca	aggtttttcga	ttgctcccag	cagcctggac
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1081	cgtccccggg	aaaaagaaga	accagtgggt	cgagagacag	gtgaggtggg	cgactgccac
1141	ctcagtgcac	tgctgcagca	gctgcacagt	gtcaatgcct	ccaagccctc	ggagcgtggg
1201	ctggtcaggc	aagaggaggc	tgaggatcct	gcctgcatcc	ccatcttctg	ggtcagcaag
1261	tgggtgggact	attcggacaa	gtacggcctt	gggtatcagc	tctgtgataa	cagcgtgggg
1321	gtgctcttca	atgactcaac	acgcctcatc	ctctacaatg	atggtgacag	cctgcagtac
1381	atagagcgtg	acggcactga	gtcctacctc	accgtgagtt	cccatcccaa	ctccttgatg
1441	aagaagatca	ccctccttaa	atatttccgc	aattacatga	gcgagcactt	gctgaaggca
1501	ggtgccaaca	tcacgccgcg	cgaagggtgat	gagctcgccc	ggctgcccta	cctacggacc
1561	tggttccgca	cccgcagcgc	catcatcctg	cacctcagca	acggcagcgt	gcagatcaac
1621	ttcttccagg	atcacaccaa	gtcatcttgc	tgcccactga	tggcagccgt	gacctacatc
1681	gacgagaagc	gggacttccg	cacataccgc	ctgagtctcc	tggaggagta	cggctgctgc

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1741 aaggagctgg ccagccggct ccgctacgcc cgcactatgg tggacaagct gctgagctca
1801 cgctcggcca gcaaccgtct caaggcctcc taa

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