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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 Ni^{2+}-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 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.

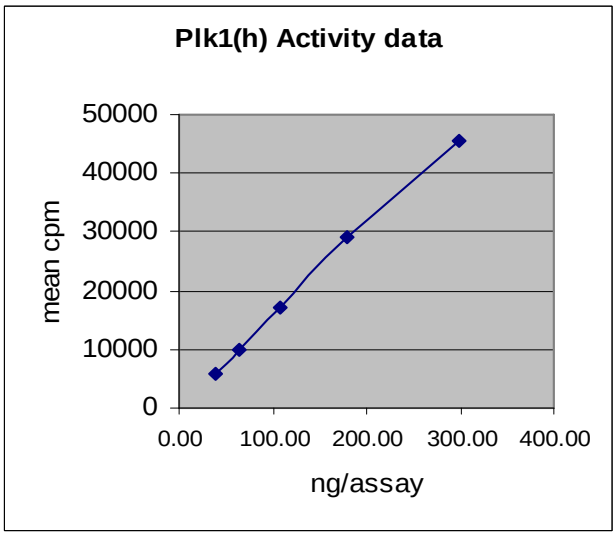
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge 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

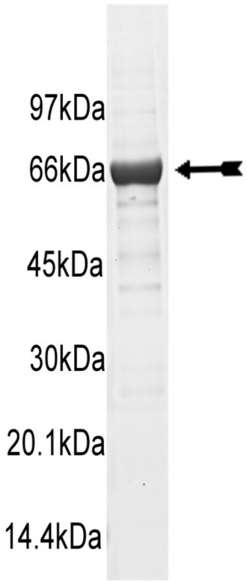
Quality Control Testing

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-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]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 dH_2O.
6. Add 10µl of diluted [γ-^{33}P]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

Protein	human Pkl1
Tags	*N*-terminal 6His
Native sequence	M8 of the recombinant protein is equivalent to M1 of human Plk1
Accession number	GenBank NM_005030

Recombinant Plk1 amino acid sequence:

```
  1 MHHHHHHMSA AVTAGKLARA PADPGKAGVP GVAAPGAPAA APPAKEIPEV LVDPRSRRRY
 61 VRGRFLGKGG FAKCFEISDA DTKEVFAGKI VPKSLLLKPH QREKMSMEIS IHRSLAHQHV
121 VGFHGFFEDN DFVFVVLELC RRRSLLELHK RRKALTEPEA RYYLRQIVLG CQYLHRNRVI
181 HRDLKLGNLF LNEDLEVKIG DFGLATKVEY DGERKKTLCG TPNYIAPEVL SKKGHSFEVD
241 VWSIGCIMYT LLVGKPPFET SCLKETYLRI KKNEYSIPKH INPVAASLIQ KMLQTDPTAR
301 PTINELLNDE FFTSGYIPAR LPITCLTIPP RFSIAPSSLD PSNRKPLTVL NKGLENPLPE
361 RPREKEEPVV RETGEVVDCH LSDMLQQLHS VNASKPSERG LVRQEEAEDP ACIPIFWVSK
421 WVDYSDKYGL GYQLCDNSVG VLFNDSTRLI LYNDGDSLQY IERDGTESYL TVSSHPNSLM
481 KKITLLKYFR NYMSEHLLKA GANITPREGD ELARLPYLRT WFRTRSAIIL HLSNGSVQIN
541 FFQDHTKLIL CPLMAAVTYI DEKRDFRTYR LSLLEEYGCC KELASRLRYA RTMVDKLLSS
601 RSASNRLKAS
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Recombinant Plk1 nucleotide sequence:

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   1 atgcaccatc accatcacca tatgagtgct gcagtgactg cagggaagct ggcacgggca
  61 ccggccgacc ctgggaaagc cggggtcccc ggagttgcag ctcccggagc tccggcggcg
 121 gctccaccgg cgaaagagat cccggaggtc ctagtggacc cacgcagccg gcggcgctat
 181 gtgcggggcc gctttttggg caagggcggc tttgccaagt gcttcgagat ctcggacgcg
 241 gacaccaagg aggtgttcgc gggcaagatt gtgcctaagt ctctgctgct caagccgcac
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 361 gtaggattcc acggcttttt cgaggacaac gacttcgtgt tcgtggtgtt ggagctctgc
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1021 cccagcaacc ggaagcccct cacagtcctc aataaaggct tggagaaccc cctgcctgag
1081 cgtccccggg aaaaagaaga accagtggtt cgagagacag gtgaggtggt cgactgccac
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1261 tgggtggact attcggacaa gtacggcctt gggtatcagc tctgtgataa cagcgtgggg
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1441 aagaagatca ccctccttaa atatttccgc aattacatga gcgagcactt gctgaaggca
1501 ggtgccaaca tcacgccgcg cgaaggtgat gagctcgccc ggctgcccta cctacggacc
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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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