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Certificate of Analysis

Plk3, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-572, 14-572-K, 14-572M
Parent Lot # D12MP022N

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 Plk3 residues 19–301, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA agarose. Purity 77% by SDS-PAGE and Coomassie blue staining. MW = 36kDa.

Specific Activity (Parent lot# D12MP022N): 1532U/mg, where one unit of Plk3, active activity is defined as 1nmol phosphate incorporated into 2mg/ml casein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.420mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 20mM β-glycerophosphate, 0.25mM sodium orthovanadate, 10mM NaF, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

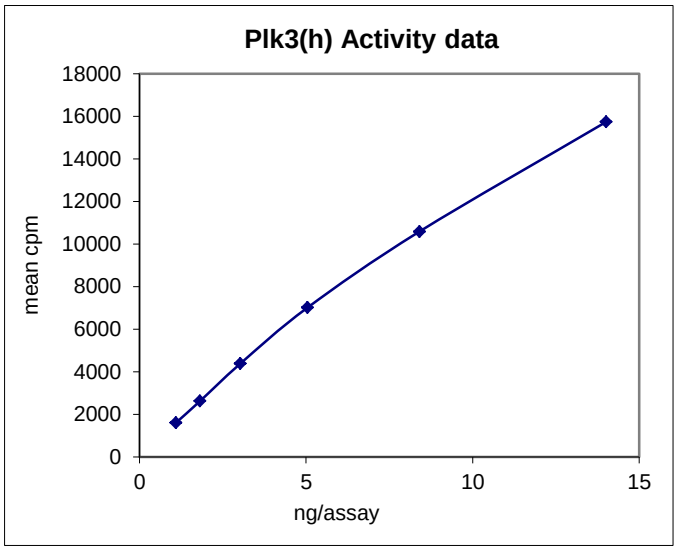
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

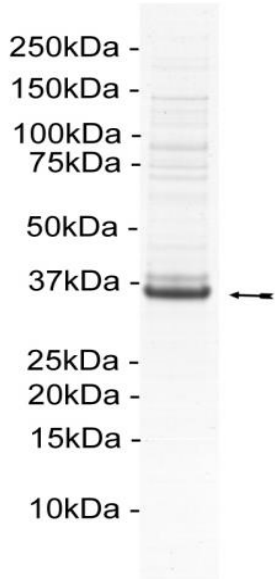
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 1–14ng of this lot of enzyme phosphorylated 2mg/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 Plk3 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 Plk3, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH 7.0, 1mM EDTA.
2. **Casein:** Use at a final assay concentration of 2mg/ml. Prepare a 20mg/ml stock and add 2.5µl of stock per assay point.
3. **Plk3, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 1mg/ml BSA. Use 1–14ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5x 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 2.5µl of **Casein**.
3. Add **2.5µl (1–14ng) Plk3, active**.
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{32}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl 3% phosphoric acid.
8. Transfer a 10µl 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 1µl of 30% phosphoric acid.

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Plk3 Sequence Information

Protein human Plk3

Tags *N*-terminal 6His

Native sequence S31 of the fusion protein is equivalent to S19 of human Plk3

Accession number GenBank NM_004073

Recombinant Plk3 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMSLGS SGRTYLKGRL LGKGGFARCY EATDTETGSA
 61 YAVKVIPQSR VAKPHQREKI LNEIELHRDL QHRHIVRFSH HFEDADNIYI FLELCSRKSL
121 AHIWKARHTL LEPEVRYYLR QILSGLKYLH QRGILHRDLK LGNFFITENM ELKVGDFGLA
181 ARLEPPEQRK KTICGTPNYV APEVLLRQGH GPEADVWSLG CVMYTLLCGS PPFETADLKE
241 TYRCIKQVHY TLPASLSLPA RQLLAAILRA SPRDRPSIDQ ILRHDFFTKG YTPDRLPISS
301 CVTVPDLTPP NPA
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Recombinant Plk3 nucleotide sequence:

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  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatgtc ccttggatcc agcggccgca cctacctcaa aggccgcttg
121 ttgggcaagg ggggcttcgc ccgctgctac gaggccactg acacagagac tggcagcgcc
181 tacgctgtca aagtcatccc gcagagccgc gtcgccaagc cgcatcagcg cgagaagatc
241 ctaaatgaga ttgagctgca ccgagacctg cagcaccgcc acatcgtgcg tttttcgcac
301 cactttgagg acgctgacaa catctacatt ttcttggagc tctgcagccg aaagtccctg
361 gcccacatct ggaaggcccg gcacaccctg ttggagccag aagtgcgcta ctacctgcgg
421 cagatccttt ctggcctcaa gtacttgcac cagcgcggca tcttgcaccg ggacctcaag
481 ttgggaaatt ttttcatcac tgagaacatg gaactgaagg tgggggattt tgggctggca
541 gcccggttgg agcctccgga gcagaggaag aagaccatct gtggcacccc caactatgtg
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781 cggcagctcc tggccgccat ccttcgggcc tcaccccgag accgcccctc tattgaccag
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901 tgcgtgacag tcccagacct gacacccccc aacccagctt ag
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Reviewed and approved by site quality representative.