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Certificate of Analysis

Wee1, active
(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-925, 14-925-K, 14-925M

Parent Lot # D12KP010N

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 6His tagged, recombinant, human Wee1 amino acids 214-end expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose.
Purity 86.3% by SDS-PAGE and Coomassie blue staining. MW = 77kDa.

Specific Activity (Parent lot# D12KP010N): 101U/mg, where one unit of Wee1, active activity is defined as 1nmol phosphate incorporated into 500µM (LSNLYHQGKFLQTFCGSPLYRRR) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.23mg/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.

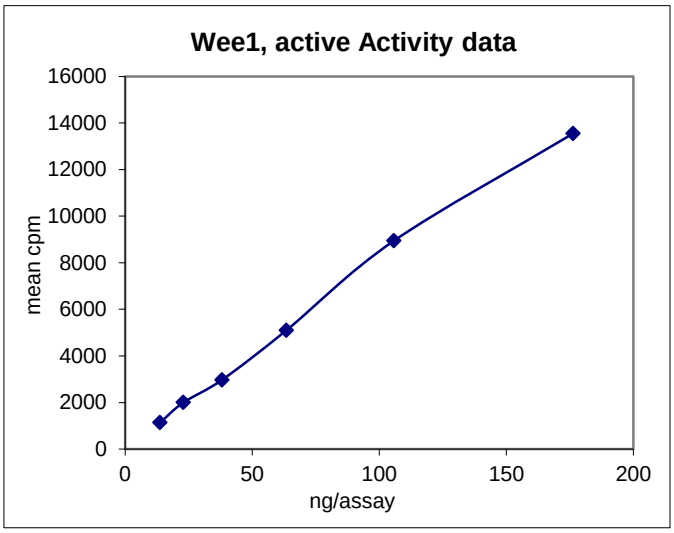
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

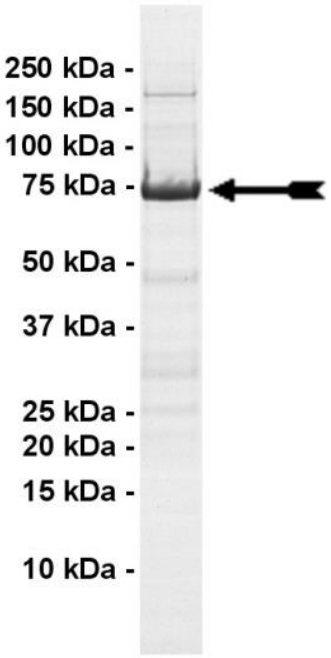
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 14–176ng of this lot of enzyme phosphorylated 500µM (LSNLYHQGKFLQTFCGSPLYRRR) 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 Wee1 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 Wee1, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 100mM Tris/HCl pH8.5, 1mM EDTA.
2. **LSNLYHQGKFLQTFCGSPLYRRR**: Use at a final assay concentration of 500µM. Prepare a 5mM stock and add 2.5µl of stock per assay point.
3. **Wee1, active:** Dilute with 20mM Tris/HCl pH8.5, 0.2mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol. Use 14–176ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[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 **(LSNLYHQGKFLQTFCGSPLYRRR)**.
3. Add **2.5µl (14–176ng) Wee1, active.**
4. Add 5µl of dH_2O.
5. Add 10µl of diluted [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 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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Wee1 Sequence Information

Protein human Wee1

Tags *N*-terminal GST and *C*-terminal 6His

Native sequence F230 of the recombinant protein is equivalent to F214 of human Wee1

Accession number GenBank NM_003390

Recombinant Wee1 amino acid sequence:

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  1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
 61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF MDTEKSGKRE
241 FDVRQTPQVN INPFTPDSLL LHSSGQCRRR KRTYWNDSCG EDMEASDYEL EDETRPAKRI
301 TITESNMKSR YTTEFHELEK IGSGEFGSVF KCVKRLDGCI YAIKRSKKPL AGSVDEQNAL
361 REVYAHAVLG QHSHVVRYFS AWAEDDHMLI QNEYCNGGSL ADAISENYRI MSYFKEAELK
421 DLLLQVGRGL RYIHSMSLVH MDIKPSNIFI SRTSIPNAAS EEGDEDDWAS NKVMFKIGDL
481 GHVTRISSPQ VEEGDSRFLA NEVLQENYTH LPKADIFALA LTVVCAAGAE PLPRNGDQWH
541 EIRQGRLPRI PQVLSQEFTE LLKVMIHPDP ERRPSAMALV KHSVLLSASR KSAEQLRIEL
601 NAEKFKNSLL QKELKKAQMA KAAAEERALF TDRMATRSTT QSNRTSRLIG KKMNRSVSLT
661 IYHHHHHH
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Recombinant Wee1 nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
 481 gttgttttat acatggaccc aatgtgcctg gatgcgttcc caaaattagt ttgtttaaa
 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
 601 tggcctttgc agggctggca agccacgttt ggtggtggcg accatcctcc aaaatcggat
 661 ctggaagttc tgttccaggg gcccgaattc atggatacag aaaaatcagg aaaaagggaa
 721 tttgatgtgc gacagactcc tcaagtgaat attaatcctt ttactccgga ttctttgttg
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 961 atcggctctg gagaatttgg ttctgtattt aagtgtgtga agaggctgga tggatgcatt
1021 tatgccatta agcgatcaaa aaagccattg gcgggctctg ttgatgagca gaacgctttg
1081 agagaagtat atgctcatgc agtgcttgga cagcattccc atgtagttcg atatttctct
1141 gcgtgggcag aagatgatca tatgcttata cagaatgaat attgtaatgg tggaagttta
1201 gctgatgcta taagtgaaaa ctacagaatc atgagttact ttaaagaagc agagttgaag
1261 gatctccttt tgcaagttgg ccgaggcttg aggtatattc attcaatgtc tttggttcac
1321 atggatataa aacctagtaa tattttcata tctcgaacct caatcccaaa tgctgcctct
1381 gaagaaggag acgaagatga ttgggcatcc aacaaagtta tgtttaaaat aggtgatctt
1441 gggcatgtaa caaggatctc cagtccacaa gttgaggagg gcgatagtcg ttttcttgca
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1501 aatgaagttt tacaggagaa ttatacccat ctaccaaaag cagatatttt tgcgcttgcc
1561 ctcacagtgg tatgtgctgc tggtgctgaa cctcttccga gaaatggaga tcaatggcat
1621 gaaatcagac agggtagatt acctcggata ccacaagtgc tttcccaaga atttacagag
1681 ttgctaaaag ttatgattca tccagatcca gagagaagac cttcagcaat ggcactggta
1741 aagcattcag tattgctgtc cgcttctaga aagagtgcag aacaattacg aatagaattg
1801 aatgccgaaa agttcaaaaa ttcactttta caaaaagaac tcaagaaagc acagatggca
1861 aaagctgcag ctgaggaaag agcactcttc actgaccgga tggccactag gtccaccacc
1921 cagagtaata gaacatctcg acttattgga aagaaaatga accgctctgt cagccttact
1981 atataccatc accatcacca tcactaa
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Reviewed and approved by site quality representative.