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Certificate of Analysis

CD45, active

(Recombinant enzyme expressed in *E. coli* cells)
Item # 14-618, 14-618-K, 14-618M
Parent Lot # 1641216

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 CD45, amino acids 598–end, expressed in *E. coli* cells. Purified using Ni^{2+}/NTA agarose.
Purity 39.2% by SDS-PAGE and Coomassie blue staining. MW = 84kDa.

Specific Activity (Parent lot# 1641216): 31108U/mg, where one unit of CD45, active activity is defined as the release of 1nmol of phosphate per minute from the phosphorylated substrate 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) at room temperature.

Formulation: 0.829mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 10% glycerol, 0.03% Brij-35, 0.1mM EGTA, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

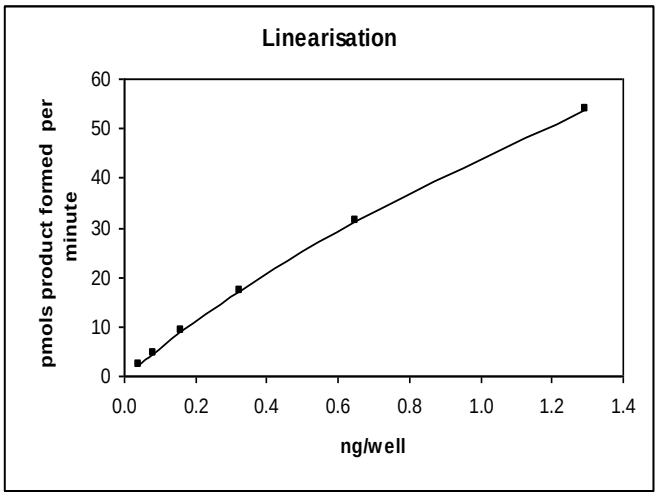
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

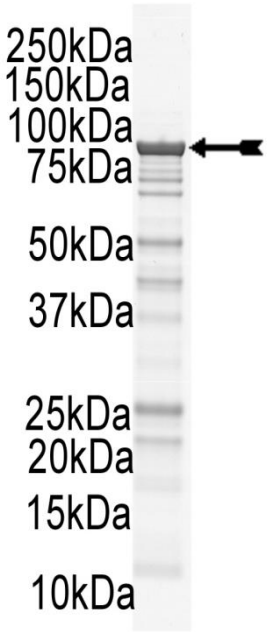
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Phosphatase Assay: 0.04–1.30ng of this lot of enzyme dephosphorylated 200µM DiFMUP in the assay described on page two. Assay background was subtracted from the actual Fluorescence Intensity (FI) to yield the results shown below. Quantification of FI was against a 6,8-difluoro-7-hydroxy-4-methylcoumarin (DiFMU) standard curve.



MS Tryptic Fingerprint: Confirmed identity as CD45 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 CD45, active.

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Phosphatase Assay Protocol

Stock Solutions:

1. **Reaction Buffer:** 60mM Hepes pH7.2, 150mM NaCl, 1mM EDTA, 0.17mM DTT, 0.83 (v/v)% glycerol, 0.017 (w/v)% BSA, 0.002% Brij-35.
2. 500µM DiFMUP (Molecular Probes Catalogue# D6567) in water.
3. 100mM sodium orthovanadate.
4. 500µM DiFMU (Molecular Probes Catalogue# D6566) in water for the calibration curve.

Assay Procedure:

1. Dilute **CD45** in reaction buffer and use 0.04–1.30ng in 15µl per assay point.
2. Add 10µl DiFMUP 500µM stock solution (200µM final assay concentration).
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5µl of 100mM sodium orthovanadate.
5. Read FI using an appropriate reader (Excitation 340nm; Emission 450nm).
6. Subtract the zero enzyme values from each FI reading and calculate the enzyme activity by conversion to nmoles product formed using a DiFMU standard calibration curve.

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CD45 Sequence Information

Protein Human CD45

Tags *N*-terminal 6His

Native sequence K24 of the recombinant protein is equivalent to K598 of human CD45

Accession number GenBank NM_002838

Recombinant CD45 amino acid sequence:

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  1 MGSSHHHHHH SSGLVPRGSH MLEKIYDLHK KRSCNLDEQQ ELVERDDEKQ LMNVEPIHAD
 61 ILLETYKRKI ADEGRLFLAE FQSIPRVFSK FPIKEARKPF NQNKNRYVDI LPYDYNRVEL
121 SEINGDAGSN YINASYIDGF KEPRKYIAAQ GPRDETVDDF WRMIWEQKAT VIVMVTRCEE
181 GNRNKCAEYW PSMEEGTRAF GDVVVKINQH KRCPDYIIQK LNIVNKKEKA TGREVTHIQF
241 TSWPDHGVPE DPHLLLKLRR RVNAFSNFFS GPIVVHCSAG VGRTGTYIGI DAMLEGLEAE
301 NKVDVYGYVV KLRRQRCLMV QVEAQYILIH QALVEYNQFG ETEVNLSELH PYLHNMKKRD
361 PPSEPSPLEA EFQRLPSYRS WRTQHIGNQE ENKSKNRNSN VIPYDYNRVP LKHELEMSKE
421 SEHDSDESSD DDSDSEEPSK YINASFIMSY WKPEVMIAAQ GPLKETIGDF WQMIFQRKVK
481 VIVMLTELKH GDQEICAQYW GEGKQTYGDI EVDLKDTDKS STYTLRVFEL RHSKRKDSRT
541 VYQYQYTNWS VEQLPAEPKE LISMIQVVKQ KLPQKNSSEG NKHHKSTPLL IHCRDGSQQT
601 GIFCALLNLL ESAETEEVVD IFQVVKALRK ARPGMVSTFE QYQFLYDVIA STYPAQNGQV
661 KKNNHQEDKI EFDNEVDKVK QDANCVNPLG APEKLPEAKE QAEGSEPTSG TEGPEHSVNG
721 PASPALNQGS
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Recombinant CD45 nucleotide sequence:

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   1 atgggcagca gccatcatca tcatcatcac agcagcggcc tggtgccgcg cggcagccat
  61 atgctcgaga aaatctatga tctacataag aaaagatcct gcaatttaga tgaacagcag
 121 gagcttgttg aaagggatga tgaaaaacaa ctgatgaatg tggagccaat ccatgcagat
 181 attttgttgg aaacttataa gaggaagatt gctgatgaag gaagactttt tctggctgaa
 241 tttcagagca tcccgcgggt gttcagcaag tttcctataa aggaagctcg aaagcccttt
 301 aaccagaata aaaaccgtta tgttgacatt cttccttatg attataaccg tgttgaactc
 361 tctgagataa acggagatgc agggtcaaac tacataaatg ccagctatat tgatggtttc
 421 aaagaaccca ggaaatacat tgctgcacaa ggtcccaggg atgaaactgt tgatgatttc
 481 tggaggatga tttgggaaca gaaagccaca gttattgtca tggtcactcg atgtgaagaa
 541 ggaaacagga acaagtgtgc agaatactgg ccgtcaatgg aagagggcac tcgggctttt
 601 ggagatgttg ttgtaaagat caaccagcac aaaagatgtc cagattacat cattcagaaa
 661 ttgaacattg taaataaaaa agaaaaagca actggaagag aggtgactca cattcagttc
 721 accagctggc cagaccacgg ggtgcctgag gatcctcact tgctcctcaa actgagaagg
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1021 gaaacagaag tgaatttgtc tgaattacat ccatatctac ataacatgaa gaaaagggat
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1261 agtgagcatg attcagatga atcctctgat gatgacagtg attcagagga accaagcaaa
1321 tacatcaatg catcttttat aatgagctac tggaaacctg aagtgatgat tgctgctcag
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1441 gttattgtta tgctgacaga actgaaacat ggagaccagg aaatctgtgc tcagtactgg
1501 ggagaaggaa agcaaacata tggagatatt gaagttgacc tgaaagacac agacaaatct
1561 tcaacttata cccttcgtgt ctttgaactg agacattcca agaggaaaga ctctcgaact
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1621 gtgtaccagt accaatatac aaactggagt gtggagcagc ttcctgcaga acccaaggaa
1681 ttaatctcta tgattcaggt cgtcaaacaa aaacttcccc agaagaattc ctctgaaggg
1741 aacaagcatc acaagagtac acctctactc attcactgca gggatggatc tcagcaaacg
1801 ggaatatttt gtgctttgtt aaatctctta gaaagtgcgg aaacagaaga ggtagtggat
1861 atttttcaag tggtaaaagc tctacgcaaa gctaggccag gcatggtttc cacattcgag
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2101 caggctgaag gttctgaacc cacgagtggc actgaggggc cagaacattc tgtcaatggt
2161 cctgcaagtc cagctttaaa tcaaggttca tag
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Reviewed and approved by site quality representative.

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