

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 Ni²⁺/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.

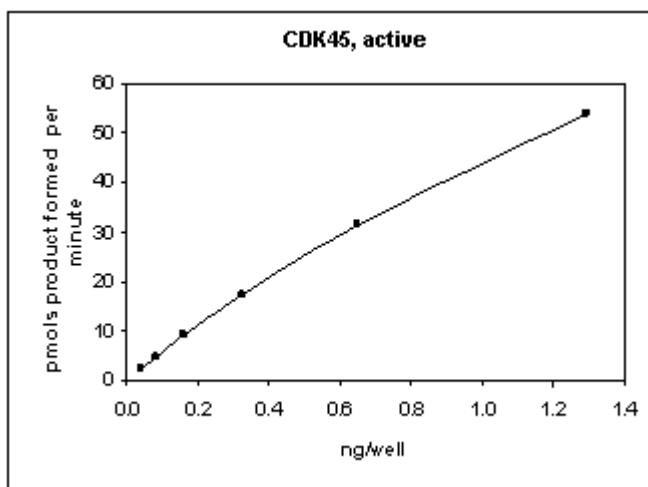
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

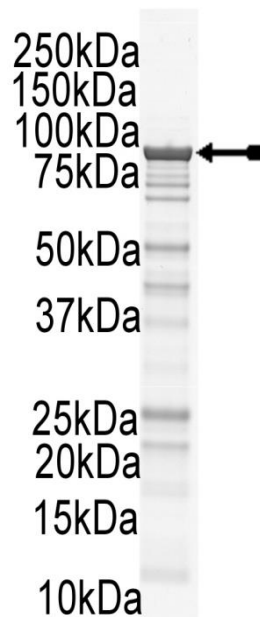
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

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. 500 μ M DiFMUP (Molecular Probes Catalogue# D6567) in water.
3. 100mM sodium orthovanadate.
4. 500 μ M 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 15 μ l per assay point.
2. Add 10 μ l DiFMUP 500 μ M stock solution (200 μ M final assay concentration).
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5 μ l 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

<u>Protein</u>	Human CD45
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	K24 of the recombinant protein is equivalent to K598 of human CD45
<u>Accession number</u>	GenBank NM_002838

Recombinant CD45 amino acid sequence:

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1  MGSSHHHHHH  SSGLVPRGSH  MLEKIYDLHK  KRSCNLDEQQ  ELVERDDEKQ  LMNVEPIHAD
61  ILLETYKRKI  ADEGRFLAE  FQSIPRVFSK  FPIKEARKPF  NQNKRYVDI  LPYDYNRVEL
121 SEINGDAGSN  YINASYIDGF  KEPRKYIAAQ  GPRDETVDDF  WRMIWEQKAT  VIVMVTRCEE
181 GNRNKCAEYW  PSMEEGTRAF  GDVVVKINQH  KRCPDYIIQK  LNIVNKKKEKA  TGREVTTHIQF
241 TSWPDHGVPE  DPHLLKLR  RVNAFSNFFS  GPIVHCSAG  VGRTGTYIGI  DAMLEGLEAE
301 NKVDVYGYVV  KLRRQRCLMV  QVEAQYILIH  QALVEYNQFG  ETEVNLSELH  PYLHNMKKRD
361 PPSEPSLEA  EFQRLPSYRS  WRTQHIGNQE  ENKSKNRNSN  VIPYDYNRVP  LKHELEMSKE
421 SEHDSDESSD  DDSDEEPSK  YINASFIMSY  WKPEVMIAAQ  GPLKETIGDF  WQMIFQRKVK
481 VIVMLTELKH  GDQEICAQYW  GEGKQTYGDI  EVDLKDTDKS  STYTLRVFEL  RHSKRKDSRT
541 VYQYQYTNWS  VEQLPAEPKE  LISMIQVVKQ  KLPQKNSSEG  NKHHKSTPLL  IHC RDGSQQT
601 GIFCALLNLL  ESAETEEVD  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
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121  gagcttggtg  aaagggatga  tgaaaaaca  ctgatgaatg  tggagccaat  ccatgcagat
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301  aaccagaata  aaaaccgtta  tgttgacatt  cttccttatg  attataaccg  tgttgaactc
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721  accagctggc  cagaccacgg  ggtgcctgag  gatcctcact  tgctcctcaa  actgagaagg
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961  caagtagagg  cccagtacat  cttgatccat  caggctttgg  tggaaataca  tcagtttggg
1021 gaaacagaag  tgaattgtc  tgaattacat  ccatatctac  ataactgaa  gaaaagggat
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1441 gttattgtta  tgctgacaga  actgaaacat  ggagaccagg  aatctgtgc  tcagtactgg
1501 ggagaaggaa  agcaaacata  tggagatatt  gaagttgacc  tgaagacac  agacaaatct
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1621 ggtaccagt accaatatac aaactggagt gtggagcagc ttcctgcaga acccaaggaa
1681 ttaatctcta tgattcaggt cgtcaaaca aaacttccc agaagaattc ctctgaaggg
1741 aacaagcatc acaagagtac acctctactc attcactgca gggatggatc tcagcaaacg
1801 ggaatatttt gtgctttggt aaatctctta gaaagtgcgg aaacagaaga ggtagtggat
1861 atttttcaag tggtaaaagc tctacgcaa gctaggccag gcatggtttc cacattcgag
1921 caatatcaat tcctatatga cgtcattgcc agcacctacc ctgctcagaa tggacaagta
1981 aagaaaaaca accatcaaga agataaaatt gaatttgata atgaagtgga caaagtaaag
2041 caggatgcta attgtgttaa tccacttggg gccccagaaa agctccctga agcaaaggaa
2101 caggctgaag gttctgaacc cacgagtggc actgaggggc cagaacattc tgtcaatggt
2161 cctgcaagtc cagctttaa tcaaggttca tag
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