

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PKM zeta [1 – 409]

<u>Enzyme description:-</u>	PKM zeta [1 – 409]
<u>Alternative Name:-</u>	PKC zeta (PRKCZ) transcript variant 2
<u>Clone number:-</u>	DU 43252
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) tag
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose

Calculated molecular mass:-

Monoisotopic 51, 401.37 daltons
Average Mass 51, 433.97 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 4.78

Purity:- 80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -70 °C

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

ERM RPRKRQGSVRRRV

Final concentration: 30 μM

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Clone Data Sheet

PKM zeta [1 – 409]

Protein PKM zeta [1 - 409]

Clone number DU 43252

Species Human

Accession number NM_001033581

Tags N-terminal His(6)

**Baculovirus
expressed protein**

MSYYHHHHHDYDIPTTENLYFQGAMGSGIQRPSTSTSSLVAAAMD
SVMPSEQEPPVDDKNEDADLPSEETDGIAYISSSRKHDSIKDDSED
LKPVIDGMDGIKISQGLGLQDFDLIRVIGRGSYAKVLLVRLKKN
QIYAMKVVKELVHDDDEDIDWVQTEKHVFEQASSNPFLVGLHSCF
QTTSRLFLVIEYVNGGDLFMHQRQRKLPEEHARFYAAEICIALN
FLHERGIIYRDLKLDNVLLDADGHIKLTGYMCKEGLGPGDTTST
FCGTPNYIAPEILRGEYGFSDVWWALGVLMFEMMAGRSPFDIIT
DNPDMNTEDYLFQVILEKPIRIPRFLSVKASHVLKGFLNKDPKER
LGCRPQTGFSDIKSHAFFRSIDWDLLEKKQALPPFQPQITDDYGL
DNFDTQFTSEPVQLTPDDEDAIKRIDQSEFEGFEYINPLLLSTEE
SV

Native sequence Amino acids M1 – V409 of human PKM zeta.
Residue M44 of the fusion protein is equivalent to M1 of the native
enzyme. The His(6) tag is located at residues 5 - 10.

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Not1* sites of pFB-HTb

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Nucleotide
Sequence of Insert

gcggccgcgATGGATTCTGTCATGCCTTCCCAAGAGCCTCCAGTA
GACGACAAGAACGAGGACGCCGACCTTCCTTCCGAGGAGACAGAT
GGAATTGCTTACATTTTCTCATCCCGGAAGCATGACAGCATTAAA
GACGACTCGGAGGACCTTAAGCCAGTTATCGATGGGATGGATGGA
ATCAAAATCTCTCAGGGGCTTGGGCTGCAGGACTTTGACCTAATC
AGAGTCATCGGGCGCGGGAGCTACGCCAAGGTTCTCCTGGTGC GG
TTGAAGAAGAATGACCAAATTTACGCCATGAAAGTGGTGAAGAAA
GAGCTGGTGCATGATGACGAGGATATTGACTGGGTACAGACAGAG
AAGCACGTGTTTGGAGCAGGCATCCAGCAACCCCTTCCTGGTCCGA
TTACACTCCTGCTTCCAGACGACAAGTCGGTTGTTCTGGTCATT
GAGTACGTCAACGGCGGGGACCTGATGTTCCACATGCAGAGGCAG
AGGAAGCTCCCTGAGGAGCACGCCAGGTTCTACGCGGCCGAGATC
TGCATCGCCCTCAACTTCTGCACGAGAGGGGGATCATCTACAGG
GACCTGAAGCTGGACAACGTCTCTCCTGGATGCGGACGGGCACATC
AAGCTCACAGACTACGGCATGTGCAAGGAAGGCCCTGGGCCCTGGT
GACACAACGAGCACTTTCTGCGGAACCCCGAATTACATCGCCCC
GAAATCCTGCGGGGAGAGGAGTACGGGTTACGCGTGGACTGGTGG
GCGCTGGGAGTCTCATGTTTGGAGATGATGGCCGGGCGCTCCCCG
TTCGACATCATCACCGACAACCCGGACATGAACACAGAGGACTAC
CTTTTCCAAGTGATCCTGGAGAAGCCCATCCGGATCCCCCGGTTT
CTGTCCGTCAAAGCCTCCCATGTTTTAAAAGGATTTTTTAAATAAG
GACCCCAAAGAGAGGCTCGGCTGCCGGCCACAGACTGGATTTTCT
GACATCAAGTCCCACGCGTTCTTCCGCAGCATAGACTGGGACTTG
CTGGAGAAGAAGCAGGCGTCCCTCCATTCCAGCCACAGATCACA
GACGACTACGGTCTGGACAACCTTTGACACACAGTTTACCAGCGAG
CCCGTGCAGCTGACCCAGACGATGAGGATGCCATAAAGAGGATC
GACCAGTCAGAGTTCGAAGGCTTTGAGTATATCAACCCATTATTG
CTGTCCACCGAGGAGTCGGTGTGAgcggccgc