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Standard Operating Procedure

Preparation of active PIM3 [2 - 326]

Enzyme description:- PIM3 [2 - 326]

Clone number:- DU 1450

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni²⁺-NTA agarose

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic 39,462.08 daltons

Average Mass 39,486.97 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.92

Purity:- > 85 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

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

Storage temperature:- -70 °C [Long term stability to be determined]

Assay:- Standard filter binding assay

Assay buffer:-

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

Substrate:-

[RSRHSSYPAGT] residues 107 – 117 of mouse BAD

Final concentration: 300 μM

Specific activity range:- To be determined

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Clone Data Sheet - PIM3 [2 - 326]

Protein PIM3 [2 - 326]

Clone number DU 1450

Species Human

Accession number Q86V86

Tags N-terminal His(6)

Baculovirus expressed protein MSYYHHHHHDYDIPTTENLYFQGAMGIRNSLLSKFGSLAHLCPGGVD
HLPVKILQPAKADKESFEKAYQVGAVLGSGGFGTVYAGSRIADGLPVAV
KHVVKERVTEWGS LGGATVPLEVVLLRKVGAAGGARGVIRLLDWFERP
GFLLVLERPEPAQDLDFDITERGALDEPLARRFFAQVLA AVRHCHSCGV
VHRDIKDENLLVDLRSGELKLIDFGSGALLKDTVYTFDGT RVYSPPEW
IRYHRYHGRSATVWSLGVLLYDMVCGDIPFEQDEEILRGLLFRRRVSP
ECQQLIRWCLSLRPSERPSLDQIAAHPWMLGADGGAPESCDLRLCTLDP
DDVASTTSSSESL

Native sequence Amino acids L2 – L326 (end) of human PIM3.
Residue L32 of the fusion protein is equivalent to L2 of the native enzyme. The His(6) tag is located at residues 5 – 10.

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Eco*R1 and *Eco*R1 site in pFastBAC HTc

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**Complete
Nucleotide
Sequence**

ATGTCGTA
ACTACCAT
CACCATCA
CCATCACG
ATTA
CGATATCC
CAACGACC
GAAAACCT
GTATTTTC
AGG
GCGCCATG
GGGATCCG
GAATTCCT
GCTCTCCAA
GTTTCGGCT
CCCTGGCG
CACCTCTG
CGGGCCCG
G
CGGCGTGG
ACCACCTC
CCGGTGA
AGATCCTG
CA
GCCAGCCA
AAGGCGG
ACAAGGAG
AGCTTCG
GAGA
AGGCGTAC
CAGGTGGG
CGCCGTG
CTGGGTAG
C
GGCGGCTT
CGGCACGG
TCTACGCG
GGTAGCCG
C
ATCGCCGAC
GGGCTCCC
GGTGGCTG
TGAAGCAC
GTGGTGA
AAGGAGCG
GGTGACCG
AGTGGGGC
AG
CCTGGGGC
GGCGGACC
GTGCCCTG
GAGGTGGT
GCTGCTGC
GAAGGTGG
GCGCGGGC
GGCGGGC
GGCG
CGCGCGGC
GTCATCCG
CCTGCTGG
ACTGGTTCC
G
AGCGGCCCG
ACGGCTTC
CTGCTGGT
GCTGGAGC
GGCCCGAG
CCGGCGC
AGGACCTC
TTCGACTT
TA
TCACGGAG
CGCGGGC
GCCCTGG
ACGAGCCG
CTG
GCGCGCCG
CTTCTTCG
CGCAGGTG
CTGGCCGCC
GTGCGCC
ACTGCCAC
AGCTGCG
GGGTGCGT
GCAC
CGCGACAT
TAAGGACG
AAAATCTG
CTTGTGGAC
CTGCGCTC
CGGAGAG
CTCAAGCT
CATCGACT
TC
GGTTCGGG
TGGCTGCT
CAAGGAC
ACGGTCTAC
ACCGACTT
CGACGGC
ACCCGAGT
GTACAGCC
CC
CCGGAGTGG
ATCCGCTA
CCACCGCT
ACCACGGG
CGCTCGGC
ACCCTGTG
GGTCGCTG
GGCGTGCT
T
CTCTACGAT
ATGGTGTG
TGGGGAC
ATCCCCTTC
G
AGCAGGAC
GAGGAGAT
CCTCCGAG
GCCGCCTG
C
TCTTCCGG
AGGAGGGT
CTCTCCAG
AGTGCCAG
C
AGCTGATC
CGGTGGT
GCCTGTCC
CTGCGGCC
CT
CAGAGCGG
CCGTCGCT
GGATCAGAT
TGCGGCC
C
ATCCCTGG
ATGCTGGG
GGCTGACG
GGGGCGCC
C
CGGAGAGCT
GTGACCTG
CGGCTGTG
CACCCTCG
A
ACCCTGAT
GACGTGGC
CAGCACCAC
GTCCAGCA
G
GCGAGAGCT
TGTGA