University of Dundee

Standard Operating Procedure

Preparation of active PIM1 [2 - 313]

Enzyme description:- PIM1 [2 - 313]

Clone number:- DU 1449

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 2 mg/L

Calculated molecular mass:-
Monoisotopic 39, 284.87 daltons
Average Mass 39, 309.66 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.86

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 -PIM1 [2 - 313]

Protein	PIM1 [2 - 313]
Clone number	DU 1449
Species	Human
Accession number	NM_002648
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGIRNS**LLSKINSLAHLRAAPCND LHATKLAPGKEKEPLESQYQVGPLLGSGGFGSVYSGIRVSDNLPVAIKH VEKDRISDWGELPNGTRVPMEVVLLKKVSSGFSGVIRLLDWFERPDSFV LILERPEPVQDLFDFITERGALQEELARSFFWQVLEAVRHCHNCGVLHR DIKDENILIDLNRGELKLIDFGSGALLKDTVYTDFDGTRVYSPPEWIRY HRYHGRSAAVWSLGILLYDMVCGDIPFEHDEEIIRGQVFFRQRVSSECQ HLIRWCLALRPSDRPTFEEIQNHPWMQDVLLPQETAEIHLHSLSPGPSK**
Native sequence	Amino acids L2 – K313 (end) of human PIM1. 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

Complete Nucleotide Sequence

ATGTCGTACTACCATCACCATCACCATCACGATTA
CGATATCCCAACGACCGAAAACCTGTATTTTCAGG
GCGCCATGGGGATCCGGAATTCC**CTCTTGTCCAAA**
ATCAACTCGCTTGCCCACCTGCGCGCCGCGCCCT
GCAACGACCTGCACGCCACCAAGCTGGCGCCCG
GCAAGGAGAAGGAGCCCCTGGAGTCGCAGTAC
CAGGTGGGCCCGCTACTGGGCAGCGGCGGCTTC
GGCTCGGTCTACTCAGGCATCCGCGTCTCCGAC
AACTTGCCGGTGGCCATCAAACACGTGGAGAAG
GACCGGATTTCCGACTGGGGAGAGCTGCCTAAT
GGCACTCGAGTGCCCATGGAAGTGGTCCTGCTG
AAGAAGGTGAGCTCGGGTTTCTCCGGCGTCATT
AGGCTCCTGGACTGGTTCGAGAGGCCCGACAGT
TTCGTCCTGATCCTGGAGAGGCCCGAGCCGGTG
CAAGATCTCTTCGACTTCATCACGGAAAGGGGA
GCCCTGCAAGAGGAGCTGGCCCGCAGCTTCTTC
TGGCAGGTGCTGGAGGCCGTGCGGCACTGCCAC
AACTGCGGGGTGCTCCACCGCGACATCAAGGAC
GAAAACATCCTTATCGACCTCAATCGCGGCGAG
CTCAAGCTCATCGACTTCGGGTCGGGGGCGCTG
CTCAAGGACACCGTCTACACGGACTTCGATGGG
ACCCGAGTGTATAGCCCTCCAGAGTGGATCCGC
TACCATCGCTACCATGGCAGGTCGGCGGCAGTC
TGGTCCCTGGGGATCCTGCTGTATGATATGGTG
TGTGGAGATATTCCTTTCGAGCATGACGAAGAG
ATCATCAGGGGCCAGGTTTTCTTCAGGCAGAGG
GTCTCTTCAGAATGTCAGCATCTCATTAGATGGT
GCTTGGCCCTGAGACCATCAGATAGGCCAACCT
TCGAAGAAATCCAGAACCATCCATGGATGCAAG
ATGTTCTCCTGCCCCAGGAAACTGCTGAGATCCA
CCTCCACAGCCTGTCGCCGGGGCCCAGCAAATA
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