

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PIM1 [2 - 313]

<u>Enzyme description:-</u>	PIM1 [2 - 313]
<u>Clone number:-</u>	DU 1449
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	2 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	39, 284.87 daltons
Average Mass	39, 309.66 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	5.86
<u>Purity:-</u>	> 85 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
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.	
<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
<u>Assay:-</u>	Standard filter binding assay
<u>Assay buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc	
<u>Substrate:-</u>	
[RSRHSSYPAGT] residues 107 – 117 of mouse BAD	
Final concentration:	300 µM
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

PIM1 [2 - 313]

<u>Protein</u>	PIM1 [2 - 313]
<u>Clone number</u>	DU 1449
<u>Species</u>	Human
<u>Accession number</u>	NM_002648
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHHHDYDIPTTENLYFQGAMGIRNSLLSKINSLAHLRAAPCND LHATKLAPGKEKEPLESQYQVGPLLGGSGFGSVYSGIRVSDNLPVAIKH VEKDRISDWGELPNGTRVPMEVVLLKKVSSGFSGVIRLLDWFERPDSFV LILERPEPVQDLDFITERGALQEELARSFFWQVLEAVRHCHNCGVLHR DIKDENILIDLNRGELKLIDFGSGALLKDTVYTDGTRVYSPPEWIRY HRYHGRSAAVWSLGILLYDMVCGDIPFEHDEEIIRGQVFFRQVSSECQ HLIRWCLALRPDRPTFEEIQNHPWMQDVLLPQETAIEIHLHSLSPGPSK
<u>Native sequence</u>	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.
<u>Protease cleavage</u>	rTEV (ENLYFQG) residues 18 - 24
<u>Cloning sites</u>	<i>Eco</i> R1 and <i>Eco</i> R1 site in pFastBAC HTc

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Complete Nucleotide Sequence

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGA
CCGAAAACCTGTATTTTCAGGGCGCCATGGGGATCCGGAATTCCCTCTT
GTCCAAAATCAACTCGCTTGCCACCTGCGCGCCGCGCCCTGCAACGAC
CTGCACGCCACCAAGCTGGCGCCCGGCAAGGAGAAGGAGCCCCTGGAGT
CGCAGTACCAGGTGGGCCCCTACTGGGCAGCGGCGGCTTCGGCTCGGT
CTACTCAGGCATCCGCGTCTCCGACAACTTGCCGGTGGCCATCAAACAC
GTGGAGAAGGACCGGATTTCCGACTGGGGAGAGCTGCCTAATGGCACTC
GAGTGCCCATGGAAGTGGTCCTGCTGAAGAAGGTGAGCTCGGGTTTCTC
CGGCGTCATTAGGCTCCTGGACTGGTTCGAGAGGCCCGACAGTTTCGTC
CTGATCCTGGAGAGGCCCGAGCCGGTGCAAGATCTCTTCGACTTCATCA
CGGAAAGGGGAGCCCTGCAAGAGGAGCTGGCCCGCAGCTTCTTCTGGCA
GGTGCTGGAGGCCGTGCGGCACTGCCACAACCTGCGGGGTGCTCCACCGC
GACATCAAGGACGAAAACATCCTTATCGACCTCAATCGCGGCGAGCTCA
AGCTCATCGACTTCGGGTCGGGGGCGCTGCTCAAGGACACCGTCTACAC
GGACTTCGATGGGACCCGAGTGTATAGCCCTCCAGAGTGGATCCGCTAC
CATCGCTACCATGGCAGGTGCGCGGCAGTCTGGTCCCTGGGGATCCTGC
TGTATGATATGGTGTGTGGAGATATTCTTTTCGAGCATGACGAAGAGAT
CATCAGGGGCCAGGTTTTCTTCAGGCAGAGGGTCTCTTCAGAATGTCAG
CATCTCATTAGATGGTGCTTGCCCTGAGACCATCAGATAGGCCAACCT
TCGAAGAAATCCAGAACCATCCATGGATGCAAGATGTTCTCCTGCCCCA
GGAACTGCTGAGATCCACCTCCACAGCCTGTCGCCGGGGGCCAGCAAA
tag