Division of Signal Transduction Therapy

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:-	Ni^{2+}-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

Division of Signal Transduction Therapy

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

```
MSYYHHHHHHDYDIPTTENLYFQGAMGIRNSLLSKFGSLAHLCGPGGVD
HLPVKILQPAKADKESFEKAYQVGAVLGSGGFGTVYAGSRIADGLPVAV
KHVVKERVTEWGSLGGATVPLEVVLLRKVGAAGGARGVIRLLDWFERPD
GFLLVLERPEPAQDLFDFITERGALDEPLARRFFAQVLAAVRHCHSCGV
VHRDIKDENLLVDLRSGELKLIDFGSGALLKDTVYTDFDGTRVYSPPEW
IRYHRYHGRSATVWSLGVLLYDMVCGDIPFEQDEEILRGRLLFRRRVSP
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

Division of Signal Transduction Therapy

Complete Nucleotide Sequence

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGA
CCGAAAACCTGTATTTTCAGGGCGCCATGGGGATCCGGAATTCC**CTGCT
CTCCAAGTTCGGCTCCCTGGCGCACCTCTGCGGGCCCGGCGGCGTGGAC
CACCTCCCGGTGAAGATCCTGCAGCCAGCCAAGGCGGACAAGGAGAGCT
TCGAGAAGGCGTACCAGGTGGGCGCCGTGCTGGGTAGCGGCGGCTTCGG
CACGGTCTACGCGGGTAGCCGCATCGCCGACGGGCTCCCGGTGGCTGTG
AAGCACGTGGTGAAGGAGCGGGTGACCGAGTGGGGCAGCCTGGGCGGCG
CGACCGTGCCCCTGGAGGTGGTGCTGCTGCGCAAGGTGGGCGCGGCGGG
CGGCGCGCGCGGCGTCATCCGCCTGCTGGACTGGTTCGAGCGGCCCGAC
GGCTTCCTGCTGGTGCTGGAGCGGCCCGAGCCGGCGCAGGACCTCTTCG
ACTTTATCACGGAGCGCGGCGCCCTGGACGAGCCGCTGGCGCGCCGCTT
CTTCGCGCAGGTGCTGGCCGCCGTGCGCCACTGCCACAGCTGCGGGGTC
GTGCACCGCGACATTAAGGACGAAAATCTGCTTGTGGACCTGCGCTCCG
GAGAGCTCAAGCTCATCGACTTCGGTTCGGGTGCGCTGCTCAAGGACAC
GGTCTACACCGACTTCGACGGCACCCGAGTGTACAGCCCCCCGGAGTGG
ATCCGCTACCACCGCTACCACGGGCGCTCGGCCACCGTGTGGTCGCTGG
GCGTGCTTCTCTACGATATGGTGTGTGGGGACATCCCCTTCGAGCAGGA
CGAGGAGATCCTCCGAGGCCGCCTGCTCTTCCGGAGGAGGGTCTCTCCA
GAGTGCCAGCAGCTGATCCGGTGGTGCCTGTCCCTGCGGCCCTCAGAGC
GGCCGTCGCTGGATCAGATTGCGGCCCATCCCTGGATGCTGGGGGCTGA
CGGGGGCGCCCCGGAGAGCTGTGACCTGCGGCTGTGCACCCTCGACCCT
GATGACGTGGCCAGCACCACGTCCAGCAGCGAGAGCTTG**tga