Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PIM2 [2 - 334]

Enzyme description:- PIM2 [2 - 334]

Clone Number:- DU 1317

Source:- Recombinant

Expression system:- *E. coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 63, 382.37 daltons
Average Mass 63, 423.31 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.82

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, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

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

Substrate:-
[RSRHSSYPAGT] residues 107 – 117 of mouse BAD
Final concentration: 300 μM

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Clone Data Sheet

PIM2 [2 - 334]

Protein	PIM2 [2 - 334]
Clone number	DU 1317
Species	Human
Accession number	U77735
Tags	N-terminal GST
E. coli expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**LTKPLQGPPAPPGT** **PTPPPGGKDREAFEAEYRLGPLLGKGGFGTVFAGHRLTDRLQVAIKVIP** **RNRVLGWSPLSDSVTCPLEVALLWKVGAGGGHPGVIRLLDWFETQEGFM** **LVLERPLPAQDLFDYITEKGPLGEGPSRCFFGQVVAAIQHCHSRGVVHR** **DIKDENILIDLRRGCAKLIDFGSGALLHDEPYTDFDGTRVYSPPEWISR** **HQYHALPATVWSLGILLYDMVCGDIPFERDQEILEAELHFPAHVSPDCC** **ALIRRCLAPKPSSRPSLEEILLDPWMQTPAEDVTPQPLQRRPCPFGLVL** **ATLSLAWPGLAPNGQKSHPMAMSQG**
Native sequence	Amino acids L2 – G334 (end) of human PIM2. Residue L232 of the fusion protein is equivalent to L2 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGPL) at residues 221 – 229
Cloning sites	*Bam*H1 and *Eco*R1 of pGEX 6P-1

Nucleotide sequence of insert

ggatccTTGACCAAGCCTCTACAGGGGCCTCCCGCGCCCCCCGGGACCC
CCACGCCGCCGCCAGGAGGCAAGGATCGGGAAGCGTTCGAGGCCGAGTA
TCGACTCGGCCCCCTCCTGGGTAAGGGGGGCTTTGGCACCGTCTTCGCA
GGACACCGCCTCACAGATCGACTCCAGGTGGCCATCAAAGTGATTCCCC
GGAATCGTGTGCTGGGCTGGTCCCCCTTGTCAGACTCAGTCACATGCCC
ACTCGAAGTCGCACTGCTATGGAAAGTGGGTGCAGGTGGTGGGCACCCT
GGCGTGATCCGCCTGCTTGACTGGTTTGAGACACAGGAGGGCTTCATGC
TGGTCCTCGAGCGGCCTTTGCCCGCCCAGGATCTCTTTGACTATATCAC
AGAGAAGGGCCCACTGGGTGAAGGCCCAAGCCGCTGCTTCTTTGGCCAA
GTAGTGGCAGCCATCCAGCACTGCCATTCCCGTGGAGTTGTCCATCGTG
ACATCAAGGATGAGAACATCCTGATAGACCTACGCCGTGGCTGTGCCAA
ACTCATTGATTTTGGTTCTGGTGCCCTGCTTCATGATGAACCCTACACT
GACTTTGATGGGACAAGGGTGTACAGCCCCCCAGAGTGGATCTCTCGAC
ACCAGTACCATGCACTCCCGGCCACTGTCTGGTCACTGGGCATCCTCCT
CTATGACATGGTGTGTGGGGACATTCCCTTTGAGAGGGACCAGGAGATT
CTGGAAGCTGAGCTCCACTTCCCAGCCCATGTCTCCCCAGACTGCTGTG
CCCTAATCCGCCGGTGCCTGGCCCCCAAACCTTCTTCCCGACCCTCACT
GGAAGAGATCCTGCTGGACCCCTGGATGCAAACACCAGCCGAGGATGTT
ACCCCTCAACCCCTCCAAAGGAGGCCCTGCCCCTTTGGCCTGGTCCTTG
CTACCCTAAGCCTGGCCTGGCCTGGCCTGGCCCCCAATGGTCAGAAGAG
CCATCCCATGGCCATGTCACAGGGAtaggaattc