MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active BMX [1 - 675]

Enzyme description:- BMX [1058 – 675]

Clone number:- DU 66233

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 105, 435.94 daltons
Average Mass 105, 503.53 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 8.18

Purity:- >75 %

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, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 deg C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 10 mM DTT, 10 mM magnesium acetate, 0. 1mM EGTA

Substrate:-
Poly Glu:Tyr (4:1) Final concentration: 1 mg/ml

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Clone Data Sheet

BMX [1 – 675]

Protein BMX [1 – 675]

Clone number DU 66233

Species Human

Accession number P51813-1

Tags N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPNSRVD**MDT**
KSILEELLLKRSQQKKKMSPNNYKERLFVLTKTNLSYYEYDKMKRGSR
KGSIEIKKIRCVEKVNLEEQTPVERQYPFQIVYKDGLLYVYASNEESR
SQWLKALQKEIRGNPHLLVKYHSGFFVDGKFLCCQQSCKAAPGCTLWE
AYANLHTAVNEEKHRVPTFPDRVLKIPRAVPVLKMDAPSSSTTLAQYD
NESKKNYGSQPPSSSTSLAQYDSNSKKIYGSQPNFNMQYIPREDFPDW
WQVRKLKSSSSSEDVASSNQKERNVNHTTSKISWEFPESSSSEEEENL
DDYDWFAGNISRSQSEQLLRQKGKEGAFMVRNSSQVGMYTVSLFSKAV
NDKKGTVKHYHVHTNAENKLYLAENYCFDSIPKLIHYHQHNSAGMITR
LRHPVSTKANKVPDSVSLGNGIWELKREEITLLKELGSGQFGVVQLGK
WKGQYDVAVKMIKEGSMSEDEFFQEAQTMMKLSHPKLVKFYGVCSKEY
PIYIVTEYISNGCLLNYLRSHGKGLEPSQLLEMCYDVCEGMAFLESHQ
FIHRDLAARNCLVDRDLCVKVSDFGMTRYVLDDQYVSSVGTKFPVKWS
APEVFHYFKYSSKSDVWAFGILMWEVFSLGKQPYDLYDNSQVVLKVSQ
GHRLYRPHLASDTIYQIMYSCWHELPEKRPTFQQLLSSIEPLREKDKH

Native sequence Amino acids M1 – H675 (end) of human BMX.
Residue M238 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Sal*1 - *Not*1 sites of pFastBac GST

Nucleotide sequence of insert

gtcgacATGGATACAAAATCTATTCTAGAAGAACTTCTTCTCAAAAGA
TCACAGCAAAAGAAGAAAATGTCACCAAATAATTACAAAGAACGGCTT
TTTGTTTTGACCAAAACAAACCTTTCCTACTATGAATATGACAAAATG
AAAAGGGGCAGCAGAAAAGGATCCATTGAAATTAAGAAAATCAGATGT
GTGGAGAAAGTAAATCTCGAGGAGCAGACGCCTGTAGAGAGACAGTAC
CCATTTCAGATTGTCTATAAAGATGGGCTTCTCTATGTCTATGCATCA
AATGAAGAGAGCCGAAGTCAGTGGTTGAAAGCATTACAAAAAGAGATA
AGGGGTAACCCCCACCTGCTGGTCAAGTACCATAGTGGGTTCTTCGTG
GACGGGAAGTTCCTGTGTTGCCAGCAGAGCTGTAAAGCAGCCCCAGGA
TGTACCCTCTGGGAAGCATATGCTAATCTGCATACTGCAGTCAATGAA
GAGAAACACAGAGTTCCCACCTTCCCAGACAGAGTGCTGAAGATACCT
CGGGCAGTTCCTGTTCTCAAAATGGATGCACCATCTTCAAGTACCACT
CTAGCCCAATATGACAACGAATCAAAGAAAAACTATGGCTCCCAGCCA
CCATCTTCAAGTACCAGTCTAGCGCAATATGACAGCAACTCAAAGAAA
ATCTATGGCTCCCAGCCAAACTTCAACATGCAGTATATTCCAAGGGAA
GACTTCCCTGACTGGTGGCAAGTAAGAAAACTGAAAAGTAGCAGCAGC
AGTGAAGATGTTGCAAGCAGTAACCAAAAAGAAAGAAATGTGAATCAC
ACCACCTCAAAGATTTCATGGGAATTCCCTGAGTCAAGTTCATCTGAA
GAAGAGGAAAACCTGGATGATTATGACTGGTTTGCTGGTAACATCTCC
AGATCACAATCTGAACAGTTACTCAGACAAAAGGGAAAAGAAGGAGCA
TTTATGGTTAGAAATTCGAGCCAAGTGGGAATGTACACAGTGTCCTTA
TTTAGTAAGGCTGTGAATGATAAAAAAGGAACTGTCAAACATTACCAC
GTGCATACAAATGCTGAGAACAAATTATACCTGGCAGAAAACTACTGT
TTTGATTCCATTCCAAAGCTTATTCATTATCATCAACACAATTCAGCA
GGCATGATCACACGGCTCCGCCACCCTGTGTCAACAAAGGCCAACAAG
GTCCCCGACTCTGTGTCCCTGGGAAATGGAATCTGGGAACTGAAAAGA
GAAGAGATTACCTTGTTGAAGGAGCTGGGAAGTGGCCAGTTTGGAGTG
GTCCAGCTGGGCAAGTGGAAGGGGCAGTATGATGTTGCTGTTAAGATG
ATCAAGGAGGGCTCCATGTCAGAAGATGAATTCTTTCAGGAGGCCCAG
ACTATGATGAAACTCAGCCATCCCAAGCTGGTTAAATTCTATGGAGTG
TGTTCAAAGGAATACCCCATATACATAGTGACTGAATATATAAGCAAT
GGCTGCTTGCTGAATTACCTGAGGAGTCACGGAAAAGGACTTGAACCT
TCCCAGCTCTTAGAAATGTGCTACGATGTCTGTGAAGGCATGGCCTTC
TTGGAGAGTCACCAATTCATACACCGGGACTTGGCTGCTCGTAACTGC
TTGGTGGACAGAGATCTCTGTGTGAAAGTATCTGACTTTGGAATGACA
AGGTATGTTCTTGATGACCAGTATGTCAGTTCAGTCGGAACAAAGTTT
CCAGTCAAGTGGTCAGCTCCAGAGGTGTTTCATTACTTCAAATACAGC
AGCAAGTCAGACGTATGGGCATTTGGGATCCTGATGTGGGAGGTGTTC
AGCCTGGGGAAGCAGCCCTATGACTTGTATGACAACTCCCAGGTGGTT
CTGAAGGTCTCCCAGGGCCACAGGCTTTACCGGCCCCACCTGGCATCG
GACACCATCTACCAGATCATGTACAGCTGCTGGCACGAGCTTCCAGAA
AAGCGTCCCACATTTCAGCAACTCCTGTCTTCCATTGAACCACTTCGG
GAAAAAGACAAGCATTGAgcggccgc