

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of BMH1 [1 - 267]

<u>Enzyme description:-</u>	BMH1 [1 – 267]
<u>Clone number:-</u>	DU 383
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	<i>E.coli</i>
<u>Tag:-</u>	N-terminal His6
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose

Calculated molecular mass:-

Monoisotopic 34, 171.53 daltons

Average Mass 34, 192.81 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.09

Purity:- >80 %

Enzyme storage buffer:-

25 mM Hepes pH 7.5, 50 % Glycerol, 1 mM DTT

Storage temperature:- -20 °C

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Clone Data Sheet

BMH1 [1 – 267]

<u>Protein</u>	BMH1 [1 – 267]
<u>Clone number</u>	DU 383
<u>Species</u>	<i>Saccharomyces cerevisiae</i>
<u>Accession number</u>	CAA46959
<u>Tags</u>	N-terminal His6
<u>Bacterially expressed protein</u>	MGGSHHHHHHGMASMTGGQQMGRTLYDDDDKDRWGSMSTSREDSVYLAK LAEQAERYEEMVENMKTVASSGQELSVEERNLLSVAYKNVIGARRASWR IVSSIEQKEESKEKSEHQVELICSYRSKIETELTKISDDILSVLDShLI PSATTGESKVFYYKMKGDYHRYLAEFSSGDAREKATNASLEAYKTASEI ATTELPPTHPIRLGLALNFSVFYYEIQNSPKACHLRKQAFDDAIAELD TLSEESYKDSTLIMQLLRDNLTLWTSDMSESGQAEDQQQQQQHQQQQPP AAAEGEAPK
<u>Native sequence</u>	Amino acids M1 – M267 (end) of yeast BMH1. Residue M37 of the fusion protein is equivalent to M1 of the native enzyme. The His6 tag is located at residues 5-10.
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Eco</i> R1 sites of pTRC

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Nucleotide

Sequence Of Insert

ggatccATGTCAACCAGTCGTGAAGATTCTGTGTACCTAGCCAAGTTGG
CTGAACAGGCCGAACGTTATGAAGAAATGGTCGAAAACATGAAGACTGT
TGCCTCCTCTGGCCAAGAGTTGTCGGTCGAAGAGCGTAATTTGTTGTCT
GTTGCTTATAAGAACGTTATTGGTGCTCGTCGTGCCTCTTGGAGAATTG
TTTCTTCTATTGAGCAAAAGGAGGAGTCCAAGGAGAAGTCCGAACACCA
GGTCGAGTTGATTTGTTTCGTACCGTTTGAAGATTGAGACCGAACTAACT
AAGATCTCCGACGATATTTTGTCCGTGCTAGACTCCCACTTAATTCCAT
CAGCCACCACTGGCGAGTCCAAGGTTTTCTACTATAAGATGAAGGGTGA
CTACCACCGTTATTTGGCTGAATTTTCTAGTGGCGATGCTAGAGAAAAG
GCCACAAACGCCTCTTTAGAAGCATACAAGACCGCTTCTGAAATTGCCA
CCACAGAGTTACCCCCAACTCACCCAATCCGTCTAGGTTTGGCTCTTAA
CTTCTCTGTCTTCTATTATGAAATTCAAACTCTCCAGACAAAGCCTGC
CATTTGCGCAAGCAAGCTTTTGACGACGCTATTGCTGAGTTGGACACTC
TGTCTGAAGAATCATACAAAGATAGCACACTTATCATGCAACTGCTAAG
GGACAATTTAACCTTATGGACTTCAGACATGTCCGAGTCCGGTCAAGCT
GAAGACCAACAACAACAACAACATCAGCAACAGCAGCCACCTGCTG
CCGCCGAAGGTGAAGCACCAAAGtaagaattc

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