

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of BMH2 [1 - 273]

<u>Enzyme description:-</u>	BMH2 [1 – 273]
<u>Clone number:-</u>	DU 384
<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 35, 162.03 daltons
Average Mass 35, 183.82 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.04

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

BMH2 [1 – 273]

<u>Protein</u>	BMH2 [1 – 273]
<u>Clone number</u>	DU 384
<u>Species</u>	<i>Saccharomyces cerevisiae</i>
<u>Accession number</u>	CAA46959
<u>Tags</u>	N-terminal His6
<u>Bacterially expressed protein</u>	MGGSHHHHHHGMASMTGGQQMGRITLYDDDDKDRWGSM STREDSVYLAK LAEQAERYEEMVENMKAVASSGOELSVEERNLLSVAYKNVIGARRASWR IVSSIEQKEESKEKSEHQVELIRSYRSKIETELTKISDDILSVLDSHLI PSATTGCECKVFYKMGDYHRYLAEFSSGDAREKATNSSLEAYKTASEI ATTELPPTHPIRLFLALNFSVFYYEIQNSPKACHLAKQAFDDAIAELD TLSEESYKDSTLIMQLLRDNLTLWTSDI SE SGOEDQQQQQQQQQQQQQQQ QQQAPAEQTQGEPTK
<u>Native sequence</u>	Amino acids M1 – K273 (end) of yeast BMH2. 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

ggatccATGTCCCAAACTCGTGAAGATTCTGTTTACCTAGCTAAATTAG
CTGAACAAGCCGAACGTTATGAAGAAATGGTCGAAAACATGAAGGCCGT
TGCTTCATCAGGTCAAGAGTTATCTGTCTGAAGAACGGAATCTATTGTCG
GTTGCTTACAAGAACGTCATCGGTGCTCGCCGTGCTTCATGGAGAATAG
TTTCTTCGATCGAACAAAAAGAAGAATCAAAGGAGAAATCTGAACATCA
AGTTGAATTAATCCGTTCTTACCGTTCTAAAATTGAAACTGAATTGACC
AAAATCTCTGACGACATTTTATCTGTGTTAGATTCTCATTTAATCCCTT
CTGCTACTACTGGTGAGTGTAAGTATTTTACTATAAGATGAAGGGTGA
CTACCACCGTTATTTAGCTGAATTTTCCAGCGGAGATGCAAGAGAAAAG
GCAACCAACTCCTCTTTGGAGGCTTATAAAACCGCTTCCGAAATCGCCA
CAACTGAATTGCCTCCAACCTCACCCAATTTCGTTTATTTCTAGCTTTGAA
TTTCTCCGTCTTCTATTACGAAATTCAAAACCTCTCCTGATAAGGCTTGC
CACTTGGCCAAACAAGCCTTTGATGATGCTATTGCTGAGTTAGATACTT
TATCTGAAGAATCATACAAGGATAGCACTTTGATCATGCAATTATTAAG
GGACAACTTGACCTTATGGACCTCTGATATTTCTGAATCTGGTCAAGAA
GATCAACAACAACAACAACAACAGCAACAGCAACAGCAACAACAGCAAC
AACAAGCTCCAGCTGAACAAACTCAAGGTGAACCAACCAAAtaagaatt
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