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# Certificate of Analysis

## Bmx, active

**(Recombinant enzyme expressed in Sf21 insect cells)**
**Item # 14-499, 14-499-K, 14-499M**
**Parent Lot # 1781389**

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

**Product Description:** *N*-terminal 6His-tagged, recombinant, full length, human Bmx, expressed by baculovirus in Sf21 insect cells. Purified using $Ni^{2+}$/NTA agarose. Purity 81.3% by SDS-PAGE and Coomassie blue staining. MW = 79kDa.

**Specific Activity (Parent lot# 1781389):** 325U/mg, where one unit of Bmx, active activity is defined as 1nmol phosphate incorporated into 0.1mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

**Formulation: 0.872mg/ml** of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Frozen solution.

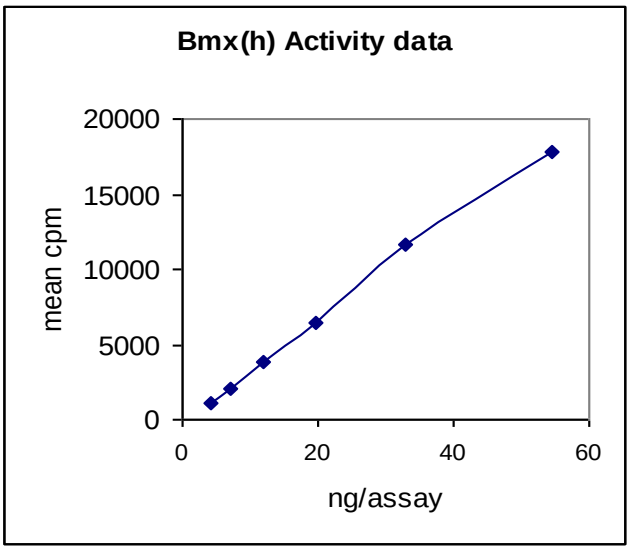
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**FOR IN VITRO RESEARCH USE ONLY**
**NOT FOR USE IN HUMANS OR ANIMALS**

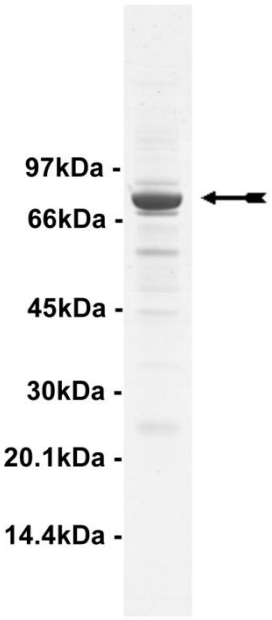
## Quality Control Testing

Kinase Assay: 4.3–55ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) (4:1) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.




MS Tryptic Fingerprint: Confirmed identity as BMX with the translated sequence listed on page three.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Bmx, active.

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**Kinase Assay Protocol**

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Poly(Glu, Tyr) (4:1):** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock. Add 2.5µl of stock per assay point.
3. **Bmx, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4.3–55ng per assay point.
4. **[γ-$^{33}$P]ATP:** 2.5 x magnesium acetate/[γ-$^{33}$P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-$^{33}$P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure: (96 well plate format):

1. Add 5µl of reaction buffer per assay to wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (4.3–55ng) Bmx, active**.
4. Add 5µl of $dH_2O$.
5. Add 10µl of diluted [γ-$^{33}$P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **Filtermat A**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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**Bmx Sequence Information**

**Protein** Human Bmx

**Tags** *N*-terminal 6His

**Native sequence** M8 of the recombinant protein is equivalent to M1 of human Bmx

**Accession number** GenBank NM_001721

***Recombinant Bmx amino acid sequence:***

```
  1 MHHHHHHMDT KSILEELLLK RSQQKKKMSP NNYKERLFVL TKTNLSYYEY DKMKRGSRKG
 61 SIEIKKIRCV EKVNLEEQTP VERQYPFQIV YKDGLLYVYA SNEESRSQWL KALQKEIRGN
121 PHLLVKYHSG FFVDGKFLCC QQSCKAAPGC TLWEAYANLH TAVNEEKHRV PTFPDRVLKI
181 PRAVPVLKMD APSSSTTLAQ YDNESKKNYG SQPPSSSTSL AQYDSNSKKI YGSQPNFNMQ
241 YIPREDFPDW WQVRKLKSSS SSEDVASSNQ KERNVNHTTS KISWEFPESS SSEEEENLDD
301 YDWFAGNISR SQSEQLLRQK GKEGAFMVRN SSQVGMYTVS LFSKAVNDKK GTVKHYHVHT
361 NAENKLYLAE NYCFDSIPKL IHYHQHNSAG MITRLRHPVS TKANKVPDSV SLGNGIWELK
421 REEITLLKEL GSGQFGVVQL GKWKGQYDVA VKMIKEGSMS EDEFFQEAQT MMKLSHPKLV
481 KFYGVCSKEY PIYIVTEYIS NGCLLNYLRS HGKGLEPSQL LEMCYDVCEG MAFLESHQFI
541 HRDLAARNCL VDRDLCVKVS DFGMTRYVLD DQYVSSVGTK FPVKWSAPEV FHYFKYSSKS
601 DVWAFGILMW EVFSLGKQPY DLYDNSQVVL KVSQGHRLYR PHLASDTIYQ IMYSCWHELP
661 EKRPTFQQLL SSIEPLREKD KH
```

***Recombinant Bmx nucleotide sequence:***

```
   1 atgcatcacc atcaccatca tatggataca aaatctattc tagaagaact tcttctcaaa
  61 agatcacagc aaaagaagaa aatgtcacca aataattaca aagaacggct ttttgttttg
 121 accaaaacaa acctttccta ctatgaatat gacaaaatga aaaggggcag cagaaaagga
 181 tccattgaaa ttaagaaaat cagatgtgtg gagaaagtaa atctcgagga gcagacgcct
 241 gtagagagac agtacccatt tcagattgtc tataaagatg ggcttctcta tgtctatgca
 301 tcaaatgaag agagccgaag tcagtggttg aaagcattac aaaaagagat aaggggtaac
 361 ccccacctgc tggtcaagta ccatagtggg ttcttcgtgg acgggaagtt cctgtgttgc
 421 cagcagagct gtaaagcagc cccaggatgt accctctggg aagcatatgc taatctgcat
 481 actgcagtca atgaagagaa acacagagtt cccaccttcc cagacagagt gctgaagata
 541 cctcgggcag ttcctgttct caaaatggat gcaccatctt caagtaccac tctagcccaa
 601 tatgacaacg aatcaaagaa aaactatggc tcccagccac catcttcaag taccagtcta
 661 gcgcaatatg acagcaactc aaagaaaatc tatggctccc agccaaactt caacatgcag
 721 tatattccaa gggaagactt ccctgactgg tggcaagtaa gaaaactgaa aagtagcagc
 781 agcagtgaag atgttgcaag cagtaaccaa aaagaaagaa atgtgaatca caccacctca
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 901 tatgactggt ttgctggtaa catctccaga tcacaatctg aacagttact cagacaaaag
 961 ggaaaagaag gagcatttat ggttagaaat tcgagccaag tgggaatgta cacagtgtcc
1021 ttatttagta aggctgtgaa tgataaaaaa ggaactgtca aacattacca cgtgcataca
1081 aatgctgaga acaaattata cctggcagaa aactactgtt ttgattccat tccaaagctt
1141 attcattatc atcaacacaa ttcagcaggc atgatcacac ggctccgcca ccctgtgtca
1201 acaaaggcca acaaggtccc cgactctgtg tccctgggaa atggaatctg ggaactgaaa
1261 agagaagaga ttaccttgtt gaaggagctg ggaagtggcc agtttggagt ggtccagctg
1321 ggcaagtgga aggggcagta tgatgttgct gttaagatga tcaaggaggg ctccatgtca
1381 gaagatgaat tctttcagga ggcccagact atgatgaaac tcagccatcc caagctggtt
1441 aaattctatg gagtgtgttc aaaggaatac cccatataca tagtgactga atatataagc
1501 aatggctgct tgctgaatta cctgaggagt cacggaaaag gacttgaacc ttcccagctc
1561 ttagaaatgt gctacgatgt ctgtgaaggc atggccttct tggagagtca ccaattcata
1621 caccgggact tggctgctcg taactgcttg gtggacagag atctctgtgt gaaagtatct
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```
1681 gactttggaa tgacaaggta tgttcttgat gaccagtatg tcagttcagt cggaacaaag
1741 tttccagtca agtggtcagc tccagaggtg tttcattact tcaaatacag cagcaagtca
1801 gacgtatggg catttgggat cctgatgtgg gaggtgttca gcctggggaa gcagccctat
1861 gacttgtatg acaactccca ggtggttctg aaggtctccc agggccacag gctttaccgg
1921 ccccacctgg catcggacac catctaccag atcatgtaca gctgctggca cgagcttcca
1981 gaaaagcgtc ccacatttca gcaactcctg tcttccattg aaccacttcg ggaaaaagac
2041 aagcattga
```

Reviewed and approved by site quality representative.