

pBLAST2-mEndo XVIII

An expression vector containing the mouse Endo XVIII open reading frame

Catalog # pbla2-mendo18

For research use only

Version # 08K24-JC

PRODUCT INFORMATION

Content:

- 20 µg of lyophilized plasmid DNA pBLAST2-mEndo XVIII
- 4 pouches of *E. coli* Fast-Media® Blas (2 for agar media, 2 for liquid media).

Storage and stability:

- Products are shipped at room temperature.
- Upon receipt, resuspend lyophilized DNA and store at -20°C. Avoid repeated freeze-thaw cycles.
- Store *E. coli* Fast-Media® Blas at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:

- Plasmid DNA was prepared using affinity column and lyophilized.
- Plasmid construct has been confirmed by restriction analysis and ORF sequencing.

GENERAL PRODUCT USE

pBLAST2 is a ready-made expression vector containing a gene of interest from the angiostatic, angiogenic, growth factor, or differentiation inhibitor family.

pBLAST2 may be used for:

Obtaining a gene to subclone into another vector. Two restriction sites flank the gene, allowing convenient excision. These restriction sites are compatible with many restriction sites contained in multiple cloning sites, thus facilitating subcloning.

Stable gene expression in mammalian cells. pBLAST2 plasmids can be used directly in transfection experiments both *in vitro* and *in vivo*. pBLAST2 plasmids contain the blasticidin resistance gene (*bsr*) driven by the CMV promoter+enhancer in tandem with the bacterial EM7 promoter. This allows the amplification of the plasmid in *E. coli* AND the selection of stable clones in mammalian cells.

pBLAST2 allows a high level of expression and secretion of the gene product: genes coding for secreted proteins are cloned with their native signal sequence. However, proteins that lack a natural signal sequence (e.g. angiostatin) are engineered for secretion by addition of the hIL-2 signal sequence.

mEndo XVIII gene may be cut out by using SgrAI and NheI enzymes.

Age I is compatible with Xma I, BspE I, NgoM IV and SgrA I.
SgrA I is compatible with Xma I, BspE I, NgoM IV, and Age I.
Nco I is compatible with BspH I and BspLU11 I.
BspH I is compatible with Nco I and BspLU11 I.
Nhe I is compatible with Xba I, Spe I, and Avr II.

PLASMID FEATURES

• **EF-1α / HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) promoter¹ and 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes for a "house keeping" gene. The promoter is stronger than CMV and is expressed at high levels in all cell cycles and lower levels during G0 phase. The promoter is also non-tissue specific; it is highly expressed in all cell types. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat² has been coupled to the EF-1α promoter to enhance stability of DNA and RNA. This modification not only increases steady state transcription, but also significantly increases translation efficiency possibly through mRNA stabilization.

• **mEndo XVIII gene:**

Intronless ORF from the ATG to the stop codon.

ORF Size (bp): 618

Cloning fragment size (bp): 674

• **SV40 polyA:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.

• **pMB1 Ori** is a minimal *E. coli* origin of replication with the same activity as the longer Ori.

• **CMV promoter & enhancer:** The Cytomegalovirus promoter allows the expression of the blasticidin resistance gene in mammalian cells.

• **Bsr (blasticidin resistance gene):** The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic Blasticidin S. The *bsr* gene is driven by the CMV promoter in tandem with the bacterial EM7 promoter. Therefore each pBLAST2 plasmid can be used to select stable mammalian cells transfectants and *E. coli* transformants.

• **Human beta-Globin polyA:** The strong human beta-globin polyadenylation (pAn) signal is placed 3' of the blasticidin. The use of beta-globin pAn minimizes interference and possible recombination events with the SV40 polyadenylation signal.

References

1- Kim et al (1990). Gene 2: 217-223.

2- Takebe et al (1988). Mol. Cell Biol. 1: 466-472.

3- Carswell et al (1989). Mol. Cell Biol. 10: 4248-4258.

METHODS

Plasmid resuspension:

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1µg/µl, resuspend the DNA in 20µl of sterile water. Store resuspended plasmid at -20°C.

Selection of bacteria with *E. coli* Fast-Media® Blas:

E. coli Fast-Media® Blas is a new, fast and convenient way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* Fast-Media® Blas is a TB (liquid) or LB (solid) based medium with blasticidin, and contains stabilizers. *E. coli* Fast-Media® Blas can be ordered separately (catalog code # fas-bl-1, fas-bl-s).

Method:

- 1- Pour the contents of a pouch into a clean borosilicate glass bottle or flask.
- 2- Add 200 ml of distilled water to the flask
- 3- Heat in a microwave on MEDIUM power setting (about 400Watts), until bubbles start appearing (approximately 3 minutes). **Do not heat a closed container. Do not autoclave Fast-Media®.**
- 4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads or gloves and care when handling.**
- 5- Reheat the media for 30 seconds and gently swirl again. Repeat as necessary to completely dissolve the powder into solution. But be careful to avoid overboiling and volume loss.
- 6- Let agar medium cool to 45°C before pouring plates. Let liquid media cool to 37°C before seeding bacteria.

Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

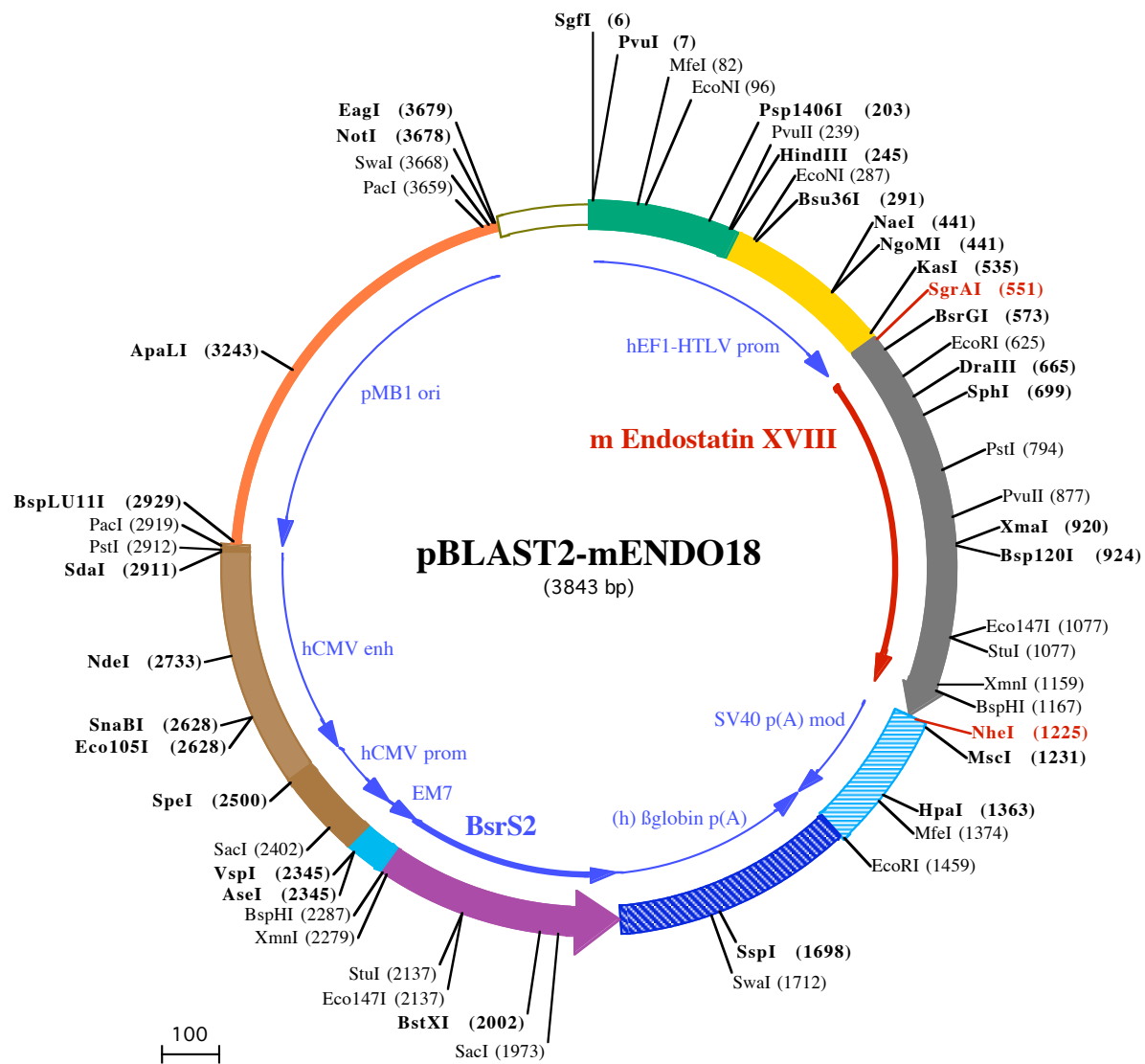
TECHNICAL SUPPORT

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PvuI (7)
SgfI (6)
MfeI (82) **EcoNI (96)**

1 GGATCTGGATCGCTCCGGTGCCCGTCAGTGGGAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCCTA
 101 GAGAAGTGGCGCGGGTAAACTGGAAAGTGATGCTGTACTGGTCCGCCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

Psp1406I (203)
HindIII (245) **Bsu36I (291)**

201 GTGAACGTTCTTTTTCGCAACGGGTTTGGCCGAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCTTACACGCGCCCGCCCTACCTGAGGCC
 301 GCCATCCACGCGGTTGAGTGCCTTCTGCCGCTCCCGCCTGTGGTGCCTCTGAAGTGCCTCCGCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

NgoMI (441)
NaeI (441)

401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCCTGCTTGTCAACTCTACGTCTTTGTTTCGTTT

KasI (535)
SgrAI (551) **BsrGI (573)**

501 TCTGTTCTGCGCGGTTACAGATCCAAGCTGTGACCGCGGCTACCTGAGATCACCGGGAAGGAGGGCCACCATGTACAGGATGCAACTCCTGTCTTGA
1► M Y R M Q L L S C

EcoRI (625)
DraIII (665) **SphI (699)**

601 TTGCACTAAGTCTTGCATTGTACGAATTCGGCTCATACTCATCAGGACTTTTCCAGCCAGTGCCTCCACCTGGTGCCAGTGAACACCCCTGTCTGGAGG
 10► I A L S L A L V T N S A H T H Q D F Q P V L H L V A L N T P L S G G

PstI (794)

701 CATGCGTGGTATCCGTGGAGCAGATTTCCAGTGCCTCCAGCAAGCCCGAGCCGTGGGGCTGTCGGGCACCTTCCGGGCTTTCTGTCTCTAGGCTGCAG
 43► M R G I R G A D F Q C F Q Q A R A V G L S G T F R A F L S S R L Q

PvuII (877)

801 GATCTCTATAGCATCGTGCCTGCTGACCGGGGCTGTGCCATCGTCAACTGAAGGACGAGGTGCTATCTCCAGCTGGGACTCCCTGTTTTCTG
 77► D L Y S I V R R A D R G S V P I V N L K D E V L S P S W D S L F S

Bsp120I (924)
XmaI (920)

901 GCTCCAGGGTCAACTGCAACCCGGGCGCCGATCTTTTCTTTTACGGCAGAGATGTCTGAGACACCCAGCCTGGCCGAGAAGAGCGTATGGCACGG
 110► G S Q G Q L Q P G A R I F S F D G R D V L R H P A W P Q K S V W H G

StuI (1077)
Eco147I (1077)

1001 CTCGGACCCAGTGGCGGAGGCTGATGGAGAGTACTGTGAGACATGGCGAACTGAACTACTGGGGCTACAGGTGAGGCTCAGGCCTCCTCCCTGCTGCAGGC
 143► S D P S G R R L M E S Y C E T W R T E T T G A T G Q A S S L L S G

BspHI (1167)

1101 AGGCTCCTGGAACAGAAAGCTGCGAGCTGCCACAACAGCTACATCGTCTGTGATTGAGAATAGCTTCATGACCTCTTTCTCCAAATAGGGCCCTCTGCC
 177► R L L E Q K A A S C H N S Y I V L C I E N S F M T S F S K •

MscI (1231)
NheI (1225)

1201 AGCTAGGGTGGCAGACAGAGCCATGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACACTAGAATGCAGTGAAAAAATGC

HpaI (1363) **MfeI (1374)**

1301 TTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAAACAGTTAAACAACAACATTCATTCTTTATGTTTCAGG

EcoRI (1459)

1401 TTCAGGGGAGGTGTGGGAGTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAACTTTAACCTCCAAT
 1501 CAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCA

SspI (1698)

1601 TGGAGTTAAGATATAGTGATTTTTCCCAAGGTTTGAAGTACTGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCACATTCCCTTTTTAGTAAAA

SwaI (1712)

1701 TATTCAGAATAATTTAAATACATCATTGCAATGAAAAATAATGTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTATAATATCCCCAGTTTA
 1801 GTAGTTGACTTAGGGAACAAAGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCTGCTGACTTGGAGGGGATGAGTT
141► • N R T Y K L P I L E

SacI (1973)

1901 CCTCAATGGTGGTTTTGACCAGCTTCCATTCTCAATGAGCACAAAGCAGTCAAGGAGCATAGTCAGAGATGAGCTCTGCACATGCCACAGGGGCT
 130► E I T T K V L K G N M E I L V F C D P A Y D S I L E R C M G C P S

BstXI (2002)

2001 GACCACCTGATGGATCTGTCCACCTCATCAGAGTGGGGTGCCTGACAGCCACAATGGTGTCAAAGTCTTCTGCCGTTGCTCACAGCAGACCCAATG
 97► V V R I S R D V E D S Y P H R V A V I T D F D K Q G N S V A S G I

StuI (2137)
Eco147I (2137)

2101 GCAATGGCTTCAGCACAGACAGTACCCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCGAGTCTTGGTCTGATGGCCGCCCGACAT
 63► A I A E A C V T V R G I Y A E I H V A S I I E G T K T R I A A G V H

BspHI (2287)
XmnI (2279)

2201 GGTGCTTGTCTCATAGAGCATGGTATCTTCTCAGTGGCGACCTCCACCAGTCCAGATCCTGCTGAGAGATGTTGAAGGCTTTCATGATGGCCCT
 30► H K N D E Y L M T I K E T A V E V L E L D Q Q S I N F T K M ◀

VspI (2345)
AseI (2345)

2301 CCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCCTAA

SacI (2402)

2401 ACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTACCGCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGAAAGTCCCCTTGATTT

SpeI (2500)

2501 ACTAGTCAAACAAACTCCATTGACGTCAATGGGGTGGAGACTTGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAAC

SnaBI (2628)
Eco105I (2628)

2600 CGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATT

NdeI (2733)

2700 TACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCCTAAATACTCCACCCATTGACGTCA

2800 ATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCAATTATTGACGTCAATGGGCGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTACCCTA

PstI (2912)
SdaI (2911) PacI (2919) BspLU11I (2929)

2900 AGTTATGTAACGCC T G C A G T T A A T T A A G A A C A T G T G A G C A A A A G G C C A G A A A A G G C C A G G A A C C G T A A A A A G G C C G C T T G C T G G C G T T T T T C C A T

2998 A G G C T C C G C C C C C C T G A C G A G C A T C A C A A A A A T C G A C G C T C A A G T C A G A G G T G G C G A A C C C G A C A G G A C T A T A A A G A T A C C A G G C G T T T C C C C T G G A A

3098 G C T C C C T C G T G C G C T C T C T G T T C C G A C C T G C C G C T T A C C G G A T A C C T G T C C G C T T T C T C C T T C G G G A A G C G T G G C G C T T T C T C A T A G C T C A G C T G

ApaLI (3243)

3198 TAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTACAGCCGACCGCTGCGCCTTATCCGGTAACTATCGT

3298 CTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGTTCT

3398 TGAAGTGGTGGCCTAACTACGGTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTG

3498 ATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTT

EagI (3679)
PacI (3659) SmaI (3668) NotI (3678)

3598 TCTACGGGGTCTGACGCTCAGTGGAAACGAAAACACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATC AGCGGCCGCAATAAAATATC

3698 TTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAATAG

3798 GCTGTCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA