

pBLAST2-hFLK1 (KDR)

An expression vector containing the human FLK1 (KDR) open reading frame

Catalog # pbla2-hflk1

For research use only

Version # 10F02-JC

PRODUCT INFORMATION

Contents:

- 20 µg of lyophilized plasmid DNA pBLAST2-hFLK1 (KDR)
- 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.

hFLK1 (KDR) gene may be cut out by using NcoI 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.

• **hFLK1 (KDR, CD309, VEGFR2) gene:**

Intronless ORF from the ATG to the stop codon.

ORF Size (bp): 4071

Cloning fragment size (bp): 4115

• **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

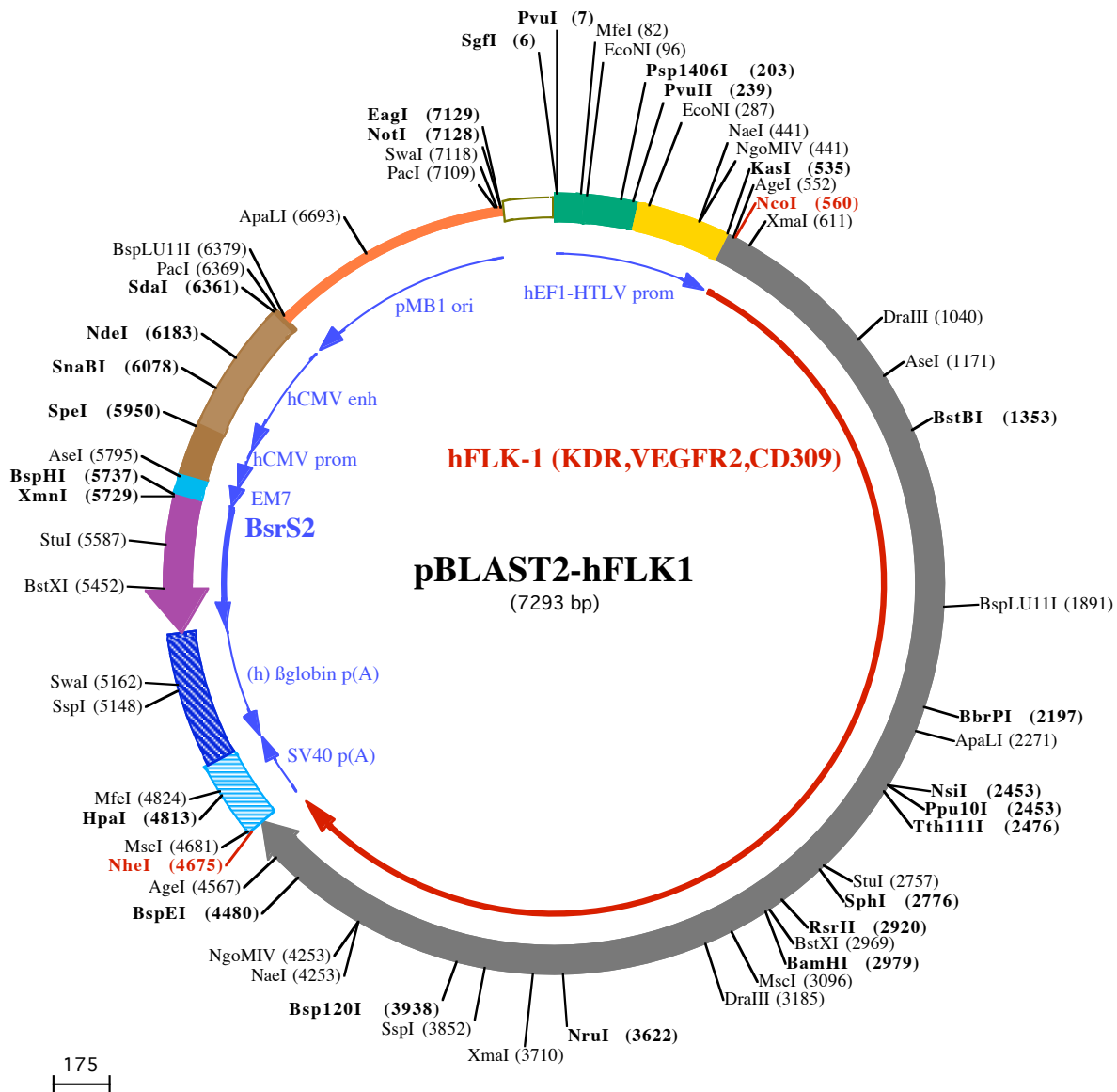
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PvuI (7)
SgfI (6) 1 GGATCTGGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCACAGTCCCGGAGAAGTTGGGGGAGGGGTGGCAATTGAACGGGTGCCTA
MfeI (82) **EcoNI (96)**
101 GAGAAGTGGCGCGGGTAAACTGGAAAGTGATGTCGTGACTGGCTCCGCCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

Psp1406I (203) **PvuII (239)** **EcoNI (287)**
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCAGAGGGCTCGCATCTCTCCTTACGCGCCCGCCCTACCTGAGGCC
301 GCCATCCACGCGGGTTGAGTCGCGTTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAAGTGCCTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

NgoMIV (441) **NaeI (441)**
401 GGGCCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCCTGCTTGTCTAACTCTACGCTTTTGTTCGTTT

KasI (535) **AgeI (552)** **NcoI (560)**
501 TCTGTTTGTCCGCGTTACAGATCCAAGCTGTGACCGGGCGCTACCTGAGATCACCGGTACCATGGAGAGCAAGTGCTGCTGGCCGTGCCCTGTGGCT
1 M E S K V L L A V A L W L

XmaI (611)
601 CTGCGTGGAGACCCGGCCGCTCTGTGGGTTGCTAGTGTCTCTTGTATCTGCCAGGCTCAGCATAACAAAAGACATACTTACAATTAAGGCTAAT
13 C V E T R A A S V G L P S V S L D L P R L S I Q K D I L T I K A N
701 ACAACTCTTCAAATTAAGTGTGAGGGGACAGAGGACTTGGACTGGCTTTGGCCCAATAATCAGAGTGGCAGTGAGCAAAGGGTGGAGGTGACTGAGTGCA
47 T T L Q I T C R G Q R D L D W L W P N N Q S G S E Q R V E V T E C
801 GCGATGGCCTCTTCTGTAAGACACTCACAAATCCAAAAGTGTGCGAAATGACTGGAGCCTACAAGTGTCTTACCAGGAAACTGACTTGGCCTCGGT
80 S D G L F C K T L T I P K V I G N D T G A Y K C F Y R E T D L A S V
901 CATTTATGTCATGTTCAAGATTACAGATCTCCATTTATTGCTTGTAGTGACCAACATGGAGTGTGTACATTACTGAGAACAAAACAAAACCTGTG
113 I Y V Y V Q D Y R S P F I A S V S D Q H G V V Y I T E N K N K T V

DraIII (1040)
1001 GTGATCCATGTCTCGGGTCCATTTCAAATCTCAACGTGTCACTTTGTGCAAGATACCCAGAAAAGAGATTTGTTCTGATGGTAACAGAATTTCTGGG
147 V I P C L G S I S N L N V S L C A R Y P E K R F V P D G N R I S W

AseI (1171)
1101 ACAGCAAGAAGGGCTTTACTATTCCAGCTACATGATCAGCTATGCTGGCATGGTCTTCTGTGAAGCAAAAATTAATGATGAAAGTTACCAGTCTATTAT
180 D S K K G F T I P S Y M I S Y A G M V F C E A K I N D E S Y Q S I M
1201 GTACATAGTTGTCTGTTGAGGGTATAGGATTTATGATGTGGTTCTGAGTCCGTCTCATGGAATGAACTATCTGTTGGAGAAAAGCTTGTCTTAAATTTG
213 Y I V V V V G Y R I Y D V V L S P S H G I E L S V G E K L V L N C

BstBI (1353)
1301 ACAGCAAGAACTGAACTAAATGTGGGATTGACTTCAACTGGGAATACCCCTTCTCGAAGCATCAGCATAAGAAAATTTGTAACCGAGACCTAAAAACCC
247 T A R T E L N V G I D F N W E Y P S S K H Q H K K L V N R D L K T
1401 AGTCTGGGAGTGAGATGAAGAAATTTTGGACACCTTAACTATAGATGGTGTAAACCCGGAGTGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCT
280 Q S G S E M K K F L S T L T I D G V T T R S D Q G L Y T C A A S S G L
1501 GATGACCAAGAAGAACAGCACATTTGTCAGGGTCTGAAACCTTTTGTGCTTTTGAAGTGGCATGGAATCTCTGGTGGAAAGCCACGGTGGGGGAG
313 M T K K N S T F V R V H E K P F V A F G S G M E S L V E A T V G E
1601 CGTGTGCAATCCCTGCAAGTACCTTGGTTACCCACCCAGAAATAAAATGGTATAAAAATGGAATACCCCTTGTGTCGAATCACACAATTAAGCGG
347 R V R I P A K Y L G Y P P P E I K W Y K N G I P L E S N H T I K A
1701 GGCATGTACTGACGATTATGGAAGTGTGAAAGAGACACAGGAAATTAACCTGTATCTTACCAATCCCATTTCAAAGGAGAAGCAGAGCCATGTGGT
380 G H V L T I M E V S E R D T G N Y T V I L T N P I S K E K Q S H V V

BspLU11I (1891)
1801 CTCTCTGGTTGTGTATGTCCACCCAGATTGGTGAGAAATCTCTAATCTCTCTGTGGATTCTACCAGTACGGCACCCTCAAACGCTGACATGTACG
413 S L V V Y V Y P P Q I G E K S L I S P V D S Y Q Y G T T Q T L T C C T
1901 GTCTATGCCATTCCTCCCGCATCACATCCACTGGTATTGGCAGTTGGAGGAGAGTGCSCAACGAGCCAGCCAAAGCTGTCTCAGTGACAAACCTAT
447 V Y A I P P P H H I H W Y W Q L E E E C A N E P S Q A V S V T N P
2001 ACCCTTGTGAAGAATGGAGAAGTGTGGAGGACTCCAGGGAGGAAATAAAATGAAGTAAATAAAAATCAATTTGCTCTAATTTGAAGGAAAAACAAAAC
480 Y P C E E W R S V E D F Q G G N K I E V N K N Q F A L I E G K N K T

BbrPI (2197)
2101 TGTAAGTACCCTTGTATCCAAGCGCAAAATGTGTCAGCTTTGTACAATGTGAAGCGGTCAACAAAGTCGGGAGAGGAGAGAGGGTGTCTCTTCCAC
513 V S T L V I Q A A N V S A L Y K C E A V N K V G R G E R V I S F H

ApaLI (2271)
2201 GTGACCAGGGGCTCTGAAATTAAGTGTGCAACCTGACATGCAGCCACTGAGCAGGAGAGCGTGTCTTTGGTGCAGTGCAGACAGATCTACGTTTGA
547 V T R G P E I T L Q P D M Q P T E Q E S V S L W C T A D R S T F E
2301 ACCTCACATGGTACAAGCTTGGCCACAGCCTCTGCCAATCCATGTGGGAGAGTTGCCACACCTGTTTGAAGAAGTGGTACTCTTTGAAATTTGAA
580 N L T W Y K L G P Q P L P I H V G E L P T P V C K N L D T L W K L N

Ppu10I (2453) **NsiI (2453)** **Tth111I (2476)**
2401 TGCCACCATGTTCTCTAATAGCACAAAATGACATTTTGTATGATGGAGCTTAAAGATGCATCCTTGCAGGACCAAGGAGACTATGTCTGGCTTCTCAAGAC
613 A T M F S N S T N D I L I M E L K N A S L Q D Q G D Y V C L A Q D
2501 AGGAAGACCAAGAAAAGACATTCGCTGGTTCAGGAGCTCACAGTCTAGAGCGTGTGGCACCCACGATCACAGGAACTGGAGAATCAGACGACAAGTA
647 R K T K K R H C V V R Q L T V L E R V A P T I T G N L E N Q T T S
2601 TTGGGAAAGCATCGAAGTCTCATGCACGGCATCTGGGAATCCCTCCACAGATCATGTGGTTTAAAGATAATGAGACCCTTGTAGAAGACTCAGGCAT
680 I G E S I E V S C T A S G N P P P Q I M W F K D N E T L V E D S G I

StuI (2757) **SphI (2776)**
2701 TGTATTGAAGGATGGAAACCGGAACCTCACTATCCGCAGAGTGAGGAAGGAGGACGAAGGCCTTACACCTGCCAGGCATGCAGTGTCTTGGCTGTGCA
713 V L K D G N R N L T I R R V R K E D E G L Y T C Q A C S V L G C A
2801 AAAGTGGAGGCATTTTTTATAATAGAAGGTGCCAGGAAAAGACGAACCTGAAATCATTATTCTAGTAGGCACGGCGGTGATTGCCATGTTCTTCTGGC
747 K V E A F F I I E G A Q E K T N L E I I I L V G T A V I A M F F W

BspHI (5737)

5701 ACCAGCTCCAGATCTGCTGAGAGATGTTGAAGTCTTCATGATGGCCCTCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAA
13 V L E L D Q Q S I N F T K M

AseI (5795)

5801 TTGTCAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACAAACGAGCTCTGCTTATATAGACCTCCACCCTACACGCTACCGCCATT

XmnI (5729)

SpeI (5950)

5901 GCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAACAAACTCCCATTGACGTCAATGGGGTGGAGACTTGGAA

SnaBI (6078)

6000 ATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCATCATGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGT

NdeI (6183)

6100 AGGAAAGTCCATAAGGTCATGTACTGGGCATAATGCCAGGGGCCATTTACCGTCATTGACGTCAATAGGGGGCTACTTGGCATATGATACACTTGA

6200 TGTACTGCCAAGTGGCAGTTTACCCTAAATACTCCACCATTGACGTCAATGGAAAGTCCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACG

SdaI (6361) PacI (6369) BspLU11I (6379)

6300 TCAATGGGCGGGGTCTGTTGGCGGTGACCCAGGCGGCCATTTACCGTAAGTTATGTAACGCC 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

6398 AGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAG

6498 GTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCCGACCTGCCGTTACCGGATACCTG

ApaLI (6693)

6598 TCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGC

6698 ACGAACCCCCGTTACGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCAAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCAC

6798 TGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATC

6898 TGGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTTGCAAGC

6998 AGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTACGGGGTCTGACGCTCAGTGAACGAAAACTCACGTTAAGGGATTTT

EagI (7129)

PacI (7109) SmaI (7118) NotI (7128)

7098 GGTATGGCTAGTTAATTAACATTTAAATC AGCGGCCGCAATAAAATATCTTTATTTTTCATTACATCTGTGTGTTGGTTTTTTTGTGTAATCGTAACTAA

7198 CATACGCTCTCCATCAAACAAACGAAACAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA