pBLAST42-hAngio

An anti-angiogenic plasmid expressing the human Angiostatin gene

Catalog # pbla-hangio

For research use only

Version # 05L21-SV

PRODUCT INFORMATION

Content:

- 20 µg of lyophilized pBLAST42-hAngio plasmid DNA.
- 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 sequencing.

GENERAL PRODUCT USE

Anti-angiogenic proteins (also called angiostatic) are utilized in cancer therapy to inhibit tumor angiogenesis, i.e. the formation of blood vessels that feed tumor cells. However, angiostatic proteins are difficult to obtain and purify. Therefore, the use of **pBLAST**, a family of plasmids expressing human or murine angiostatic genes should simplify the production of angiostatic proteins *in vitro* and *in vivo*.

pBLASTs may be used for:

- convenient production of angiostatic proteins *in vitro*
- anti-angiogenic gene therapy

pBLAST 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.

pBLAST carries a single antibiotic resistance gene, blasticidin, which allows very rapid and convenient selection of both bacteria and mammalian cell transformants.

PLASMID FEATURES

• **EF-1α / HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) promoter[1] and 5' untranslated region of the Human T-Cell Leukemia Virus (HTLV).EF-1α is an 'housekeeping' gene ubiquitously expressed in eukaryotic cells. The EF-1α promoter exhibits a strong activity, higher than viral promoters and, on the contrary to the CMV promoter, yields persistent expression of the transgene *in vivo*. The R segment and part of the U5 sequence (R-U5') of the HTLV Type 1 Long Terminal Repeat[2] 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.

• **I117** is a bacterial promoter that is spliced out as an intron in mammalian cells. Expression of the transgene in *E. coli* can be further increased by the addition of IPTG when working with bacteria that constitutively express *LacI*

• **SV40 polyA:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell et al.[3]

• **SpAn:** A synthetic polyadenylation site and a strong pause site are placed downstream of the pMB1 Ori to limit transcriptional interference between both transcriptional units. The synthetic polyA site is based on the highly efficient polyA signal of the rabbit β-globin gene[4].

• **Angiostatic gene hAngiostatin**
Size: 1155 bp.

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

• **CMV prom:** The CMV 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 SV40 promoter in tandem with the bacterial EM7 promoter. Therefore each pBLAST plasmid can be used to select stable mammalian cells transfectants and *E. coli* transformants.

• **bGh pAn:** The bovine growth hormone (bGh) polyadenylation (pAn) signal and a transcriptional pause are placed 3' of the blasticidin gene. The bGh pAn has been shown to be as efficient as SV40 and HSV1tk polyadenylation signals in many different cell types[5]. The use of bGH pAn minimizes interference and possible recombination events with the SV40 polyadenylation signal. The pause site prevents transcriptional interference and read-through.

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.
4- Levitt *et al*. (1989). Genes Dev. 7: 1019-1025.
5- Goodwin *et al*. (1992). J. Biol. Chem. 23: 16330-16334.

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 H_2O. 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-l, 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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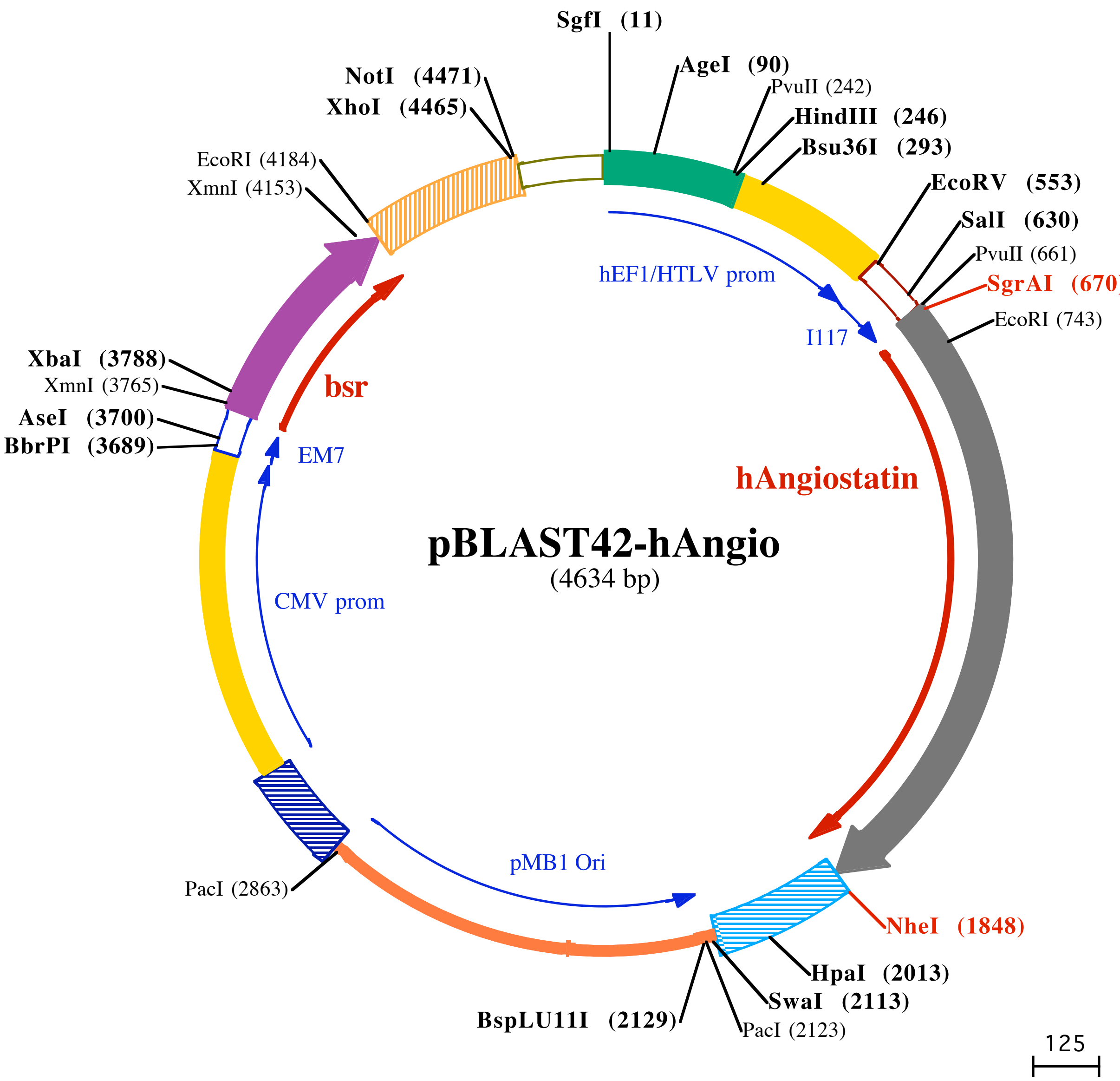

pBLAST42-hAngio
(4634 bp)
SgfI (11)
AgeI (90)
PvuII (242)
HindIII (246)
Bsu36I (293)
EcoRV (553)
SalI (630)
PvuII (661)
SgrAI (670)
EcoRI (743)
hEF1/HTLV prom
I117
hAngiostatin
NheI (1848)
HpaI (2013)
SwaI (2113)
PacI (2123)
BspLU11I (2129)
pMB1 Ori
PacI (2863)
CMV prom
EM7
bsr
BbrPI (3689)
AseI (3700)
XmnI (3765)
XbaI (3788)
XmnI (4153)
EcoRI (4184)
XhoI (4465)
NotI (4471)
125

SgfI (11) AgeI (90)

1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTA

101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

HindIII (246)

PvuII (242) Bsu36I (293)

201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT

EcoRV (553)

501 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACgtaagtgatatctactagatttatcaaaaagagtgttgacttgtgagcgctcacaa

SalI (630) PvuII (661) SgrAI (670)

601 ttgatacttagattcatcgagagggacacgtcgactactaaccttcttctctttcctacagCTGAGATCACCGGCGAAGGAGGGCCACCATGTACAGGAT

1 MetTyrArgMe

EcoRI (743)

701 GCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCACGAATTCGGTGTATCTCTCAGAGTGCAAGACTGGGAATGGAAAGAACTACAGAGGGACG

4 tGlnLeuLeuSerCysIleAlaLeuSerLeuAlaLeuValThrAsnSerValTyrLeuSerGluCysLysThrGlyAsnGlyLysAsnTyrArgGlyThr

801 ATGTCCAAAACAAAAAATGGCATCACCTGTCAAAAATGGAGTTCCACTTCTCCCCACAGACCTAGATTCTCACCTGCTACACACCCCTCAGAGGGACTGG

38 MetSerLysThrLysAsnGlyIleThrCysGlnLysTrpSerSerThrSerProHisArgProArgPheSerProAlaThrHisProSerGluGlyLeuG

901 AGGAGAACTACTGCAGGAATCCAGACAACGATCCGCAGGGGCCCTGGTGCTATACTACTGATCCAGAAAAGAGATATGACTACTGCGACATTCTTGAGTG

71 luGluAsnTyrCysArgAsnProAspAsnAspProGlnGlyProTrpCysTyrThrThrAspProGluLysArgTyrAspTyrCysAspIleLeuGluCy

1001 TGAAGAGGAATGTATGCATTGCAGTGGAGAAAACTATGACGGCAAAATTTCCAAGACCATGTCTGGACTGGAATGCCAGGCCTGGGACTCTCAGAGCCCA

104 sGluGluGluCysMetHisCysSerGlyGluAsnTyrAspGlyLysIleSerLysThrMetSerGlyLeuGluCysGlnAlaTrpAspSerGlnSerPro

1101 CACGCTCATGGATACATTCCTTCCAAATTTCCAAACAAGAACCTGAAGAAGAATTACTGTCGTAACCCCGATAGGGAGCTGCGGCCTTGGTGTTTCACCA

138 HisAlaHisGlyTyrIleProSerLysPheProAsnLysAsnLeuLysLysAsnTyrCysArgAsnProAspArgGluLeuArgProTrpCysPheThrT

1201 CCGACCCCAACAAGCGCTGGGAACTTTGTGACATCCCCCGCTGCACAACACCTCCACCATCTTCTGGTCCCACCTACCAGTGTCTGAAGGGAACAGGTGA

171 hrAspProAsnLysArgTrpGluLeuCysAspIleProArgCysThrThrProProProSerSerGlyProThrTyrGlnCysLeuLysGlyThrGlyGl

1301 AAACTATCGCGGGAATGTGGCTGTTACCGTGTCCGGGCACACCTGTCAGCACTGGAGTGCACAGACCCCTCACACACATAACAGGACACCAGAAAACTTC

204 uAsnTyrArgGlyAsnValAlaValThrValSerGlyHisThrCysGlnHisTrpSerAlaGlnThrProHisThrHisAsnArgThrProGluAsnPhe

1401 CCCTGCAAAAATTTGGATGAAAACTACTGCCGCAATCCTGACGGAAAAAGGGCCCCATGGTGCCATACAACCAACAGCCAAGTGCGGTGGGAGTACTGTA

238 ProCysLysAsnLeuAspGluAsnTyrCysArgAsnProAspGlyLysArgAlaProTrpCysHisThrThrAsnSerGlnValArgTrpGluTyrCysL

1501 AGATACCGTCCTGTGACTCCTCCCCAGTATCCACGGAACAATTGGCTCCCACAGCACCACCTGAGCTAACCCCTGTGGTCCAGGACTGCTACCATGGTGA

271 ysIleProSerCysAspSerSerProValSerThrGluGlnLeuAlaProThrAlaProProGluLeuThrProValValGlnAspCysTyrHisGlyAs

1601 TGGACAGAGCTACCGAGGCACATCCTCCACCACCACCACAGGAAAGAAGTGTCAGTCTTGGTCATCTATGACACCACACCGGCACCAGAAGACCCCAGAA

304 pGlyGlnSerTyrArgGlyThrSerSerThrThrThrThrGlyLysLysCysGlnSerTrpSerSerMetThrProHisArgHisGlnLysThrProGlu

1701 AACTACCCAAATGCTGGCCTGACAATGAACTACTGCAGGAATCCAGATGCCGATAAAGGCCCCTGGTGTTTTACCACAGACCCCAGCGTCAGGTGGGAGT

338 AsnTyrProAsnAlaGlyLeuThrMetAsnTyrCysArgAsnProAspAlaAspLysGlyProTrpCysPheThrThrAspProSerValArgTrpGluT

NheI (1848)

1801 ACTGCAACCTGAAAAAATGCTCAGGAACAGAAGCGAGTGTTGTATAGGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGA

371 yrCysAsnLeuLysLysCysSerGlyThrGluAlaSerValVal •••

1901 ATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTG

HpaI (2013)

2001 CAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGT

PacI (2123)

SwaI (2113) BspLU11I (2129)

2101 GGTAGATCCATTTAAATGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGC

2201 TCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTC

2301 CCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGG

2401 TATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTG

2501 AGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAA

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PacI (2863)

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3401 GCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAAC

3501 AACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGACGCC

BbrPI (3689) AseI (3700)

3601 ATCCACGCTGTTTTGACCTCCATAGAAGACACCGGGACCGATCCAGCCTCCGCGGCCGGGAACGGTGCATTGGAACGGACCTGCAGCACGTGTTGACAAT

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                                                                        XmnI (3765)               XbaI (3788)
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  48▸isIleGluAlaTyrIleGlyArgValThrValCysAlaGluAlaIleAlaIleGlySerAlaValSerAsnGlyGlnLysAspPheAspThrIleValAl
4001 TGTTAGACACCCTTATTCTGACGAAGTAGATAGAAGTATTCGAGTGGTAAGTCCTTGTGGTATGTGTAGGGAGTTGATTTCAGACTATGCACCAGATTGT
  81▸aValArgHisProTyrSerAspGluValAspArgSerIleArgValValSerProCysGlyMetCysArgGluLeuIleSerAspTyrAlaProAspCys
                                                            XmnI (4153)                   EcoRI (4184)
4101 TTTGTGTTAATAGAAATGAATGGCAAGTTAGTCAAAACTACGATTGAAGAACTCATTCCACTCAAATATACCCGAAATTAAAAGAATTCGCTAGAGGGCC
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4201 CTATTCTATAGTGTCACCTAAATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTC
4301 CTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGG
                                                                                 NotI (4471)
                                                                             XhoI (4465)
4401 GTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCGCAGGGCCCAATTGCTCGAGCGGCCGCAATAAAATATCTTTATTTTCATT
4501 ACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGT
4601 GCAAGTGCAGGTGCCAGAACATTTCTCTATCGAA
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