

pBLAST45-mCALR

An anti-angiogenic plasmid expressing the murine Calreticulin gene

Catalog # pbla-mcalr

For research use only

Version # 02H23-SV

PRODUCT INFORMATION

Content:

- 20 µg of lyophilized pBLAST-mCALR plasmid DNA.
- 4 pouches of *E. coli* FastMedia™ Blast (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* FastMedia™ Blas at room temperature. FastMedia™ 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 / eIF4g hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) promoter¹ and the 5' untranslated region of the translation initiation factor eIF4g. 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 eIF4g 5'UTR has been coupled to the EF-1α promoter to enhance stability of DNA and RNA. This 5'UTR acts as a translational enhancer when placed upstream of a gene, due to its ability to direct internal initiation².
- **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 gene⁴.

• **Angiostatic mCALR gene**

Size: 1251 bp

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

• **SV40 prom:** The Simian Virus 40 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 types⁵. 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- Gan, W., and Rhoads, R.E. (1996). *J. Biol. Chem.* 271: 623-626.
- 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 H₂O. Store resuspended plasmid at -20°C.

Selection of bacteria with *E. coli* FastMedia™ Blas:

E. coli FastMedia™ Blas is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* FastMedia™ Blas is a TB (liquid) or LB (solid) based medium with blasticidin, and contains stabilizers. *E. coli* FastMedia™ 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 FastMedia™.**
- 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.

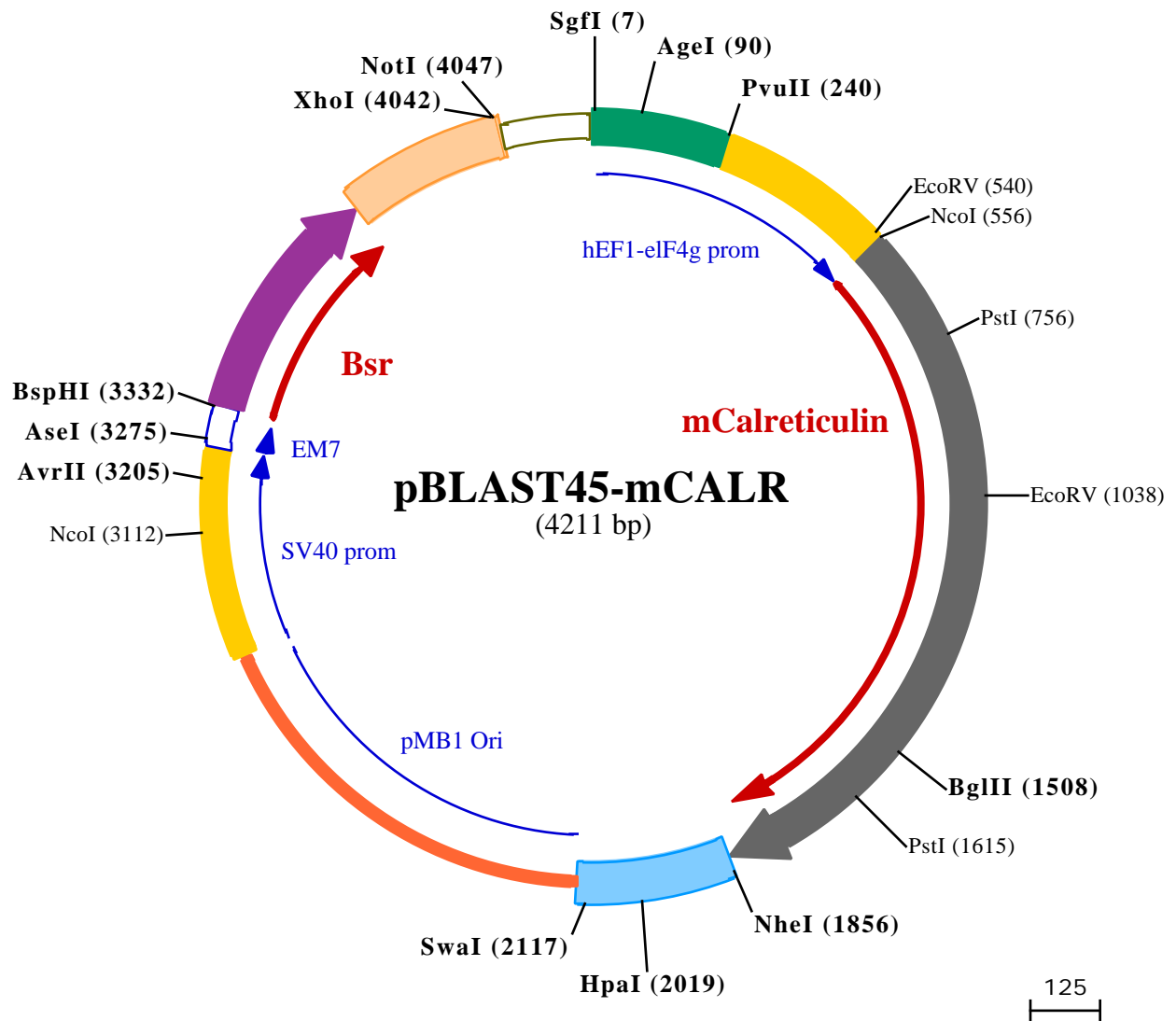
Note: Do not reheat solidified FastMedia™ as the antibiotic will be permanently destroyed by the procedure.

TECHNICAL SUPPORT

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SgfI (7)

AgeI (90)

1 GGATCTGCGATCGTCCGGTGCCCGTCAGTGGGCAGAGCCACATCGCCACAGTCCCCGAGAAGTTGGGGGGAGGGTTCGGCAATTGAACCGGTGCGCTA
101 GAGAAGGTGGCGGGGTAACACTGGGAAAGTGATGTCGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCCGC

PvuII (240)

201 GTGAACGTTCTTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGGTGGGTAGGGATGAGGGAGGGAGGGGCATTGTGATGTACAGGGCTGCTCTGTGAG
301 ATCAAGGGTCTCTTAAGGGTGGGAGCTGGGGCAGGGACTACGAGAGCAGCCAGATGGGCTGAAAGTGGAACTCAAGGGGTTTCTGGCACCTACCTACCTG
401 CTTCCCGCTGGGGGGTGGGGAGTTGGCCAGAGCTTAAAGATTGGGGCAGGGTGGAGAGGTGGGCTCTTCTGCTTCCCACTCATCTTATAGCTTTCTTT

EcoRV (540)

NcoI (556)

501 CCCCAGATCCGAATTCGAGATCCAAACCAAGGAGGAAAGGATATCACAGAGGAGACCATGGCTCTCCTTTCGGTGGCGCTCCTGCTTGGCCTCCTCGGCC
601 TGGCCGCCGAGACCTGCATCTATTTCAAAGAGCAGTCTTGGACGGAGATGCCTGGACCAACCGCTGGGTGCAATCCAAACATAAGTCCGATTTTGG
15 MetAlaLeuLeuSerValProLeuLeuLeuGlyLeuLeuGlyL
701 CAAATTTGCTCCTCAGTTCTGGCAAATTTTACGGGGACCTGGAGAAGGATAAAGGGCTGCAGACAAGCCAAGATGCCCGATTTTACGCCTGTCCGCCAAA
48 yLysPheValLeuSerSerGlyLysPheTyrGlyAspLeuGluLysAspLysGlyLeuGlnThrSerGlnAspAlaArgPheTyrAlaLeuSerAlaLys
801 TTCAACCCCTCAGCAATAAGGGCCAGACACTGGTGGTACAGTTTACCGTGAAGCATGAGCAGAATATCGACTTGGGGGGCGGCTACGTGAAGCTGTTTC
82 PheGluProPheSerAsnLysGlyGlnThrLeuValValGlnPheThrValLysHisGluGlnAsnIeAspCysGlyGlyGlyTyrValLysLeuPheP
901 CGAGTGGCTTGGACCAGAAGGACATGCATGGAGACTCAGAATATAACATCATGTTTGGTCCGGACATCTGCGGTCTGGCACCAGAAGGTTTATGTCAT
115 roSerGlyLeuAspGlnLysAspMetHisGlyAspSerGluTyrAsnIeMetPheGlyProAspIeCysGlyProGlyThrLysLysValHisValII

EcoRV (1038)

1001 CTTTAACTACAAGGCAAGAATGTGCTGATCAACAAGGATATCCGGTGTAAAGATGATGAATTCACACACCTATAACACTGATTGTGCGGCCAGACAAC
148 ePheAsnTyrLysGlyLysAsnValLeuIeAsnLysAspIeArgCysLysAspAspGluPheThrHisLeuTyrThrLeuIeValArgProAspAsn
1101 ACCTATGAGGTGAAAATTGACAACAGCCAGGTGGAGTCAAGGCTCCTTGGAGGATGATTGGGACTTCTGCCACCAAGAGATAAAGGACCCTGATGCTG
182 ThrTyrGluValLysIeAspAsnSerGlnValGluSerGlySerLeuGluAspAspTrpAspPheLeuProProLysLysIeLysAspProAspAlaA
1201 CCAAGCCGGAAGACTGGGATGAACGAGCCAAGATCGATGACCCACAGATTCCAAGCCTGAGGACTGGGACAAGCCAGAGCACATCCCTGACCCGTATGC
215 laLysProGluAspTrpAspGluArgAlaLysIeAspAspProThrAspSerLysProGluAspTrpAspLysProGluHisIeProAspProAspAl
1301 TAAGAAGCCTGAGGACTGGGATGAAGAGATGGATGGAGAGTGGGAACCACAGTATTCAAATCCTGAATACAAGGGCGAGTGGAAACCACGTCAAAAT
248 aLysLysProGluAspTrpAspGluGluMetAspGlyGluTrpGluProProValIeGlnAsnProGluTyrLysGlyGluTrpLysProArgGlnIe
1401 GACAACCCAGATTACAAGGTACCTGGATACCCAGAAATGACAACCTGAATACTCCCGGATGCAAAATATCTATGCCTATGATAGTTTTGCTGTAC
282 AspAsnProAspTyrLysGlyThrTrpIeHisProGluIeAspAsnProGluTyrSerProAspAlaAsnIeTyrAlaTyrAspSerPheAlaValI

BglIII (1508)

1501 TGGGCCTAGATCTTGGCAGGTCAAGTCTGGGACAATCTTTGACAATTTCTCCTACCAATGATGAGGCCTATGCAGAGGAGTTTGGCAATGAGACGTTG
315 euGlyLeuAspLeuTrpGlnValLysSerGlyThrIePheAspAsnPheLeuIeThrAsnAspGluAlaTyrAlaGluGluPheGlyAsnGluThrTr
1601 GGGTGTTACCAAGGCTGCAGAGAAGCAGATGAAGGACAAGCAGGATGAGGAGCAGAGGCTTAAGGAAGAAGAAGAGGACAAGAAGCGTAAAGAGGAAGAA
348 pGlyValThrLysAlaAlaGluLysGlnMetLysAspLysGlnAspGluGluGlnArgLeuLysGluGluGluGluAspLysLysArgLysGluGluGlu
1701 GAAGCTGAGATAAAGAGGATGATGATGACAGAGATGAAGATGAGGACGAAGAAGATGAGAAGGAGGAAGATGAGGAAGAATCCCTGGCCAAGCCAAGG
382 GluAlaGluAspLysGluAspAspAspArgAspGluAspGluAspGluGluAspGluLysGluGluAspGluGluGluSerProGlyGlnAlaLysA

NheI (1856)

1801 ATGAGCTGTAGAGCCACACCACCTGCCTTCAGGGCTGGACTGAGGCCTGAACACGCTAGCTCGACATGATAAGATACATTGATGAGTTTGGACAAACCA
415 spGluLeu•••
1901 CAACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACATT

HpaI (2019)

2001 ATAAGCTGCAATAAACAAGTTAACAACAACAAATTCATTTCATTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAACCTCT

SwaI (2117)

2101 ACAAATGTGGTAGATCATTTAAATGTTAATTAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTGTGCGGCTTTTTTC
2201 CATAGGCTCCGCCCCCTGACGAGCATCAAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCCTG
2301 GAAGCTCCCTCGTGCCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAATGCTCAGC
2401 CTGTAGGTATCTCAGTTCCGTTGAGTTCGTTCCGCTCAAGCTGGGCTGTGTGCAGCAACCCCGTTCCAGCCGACCGTGGCGCTTATCCGGTAACTAT
2501 CGTCTTGAGTCCAACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGT
2601 TCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTC
2701 TTGATCCGGCAAACAAACCACCGTGGTACGGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATC
2801 TTTTCTACGGGCTCTGACGCTCAGTGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAAGCTGTACACTGTGGAATGTGTGTCAGT
2901 TAGGGTGTGAAAGTCCCCAGGCTCCCCAGCAGGAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCC
3001 AGCAGGCAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCTAACTCCGCCATCCCGCCCTAACTCCGCCAGTTCCGCC

NcoI (3112)

3101 CATTCTCCGCCCATGGCTGACTAATTTTTTTTATTTATGACAGAGGCCGAGGCCGCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTG

AvrII (3205) **AseI (3275)**
 3201 GAGGCCTAGGCTTTTGCAAAAAGCTCCCGGGAGCTTGTATATCCATTTTCGGATCTGATcagCACGTGTTGACAATTAATCATCGGCATAGTATATCGGC

BspHI (3332)

3301 ATAGTATAATACGACAAGGTGAGGAATAATCATGAAGACCTTCAACATCTCTCAGCAGGATCTGGAGCTGGTGAGGTCGCCACTGAGAAGATCACCA
1▶MetLysThrPheAsnI leSerGlnGlnAspLeuGluLeuValGluValAlaThrGluLysI leThrM
 3401 TGCTCTATGAGGACAACAAGCACCATGTCGGGGCGGCCATCAGGACCAAGACTGGGGAGATCATCTCTGCTGTCCACATTGAGGCCTACATTGGCAGGGT
23▶etLeuTyrGluAspAsnLysHisHisValGlyAlaAlal leArgThrLysThrGlyGluI leI leSerAlaValHisI leGluAlaTyrI leGlyArgVa
 3501 CACTGTCTGTGCTGAAGCCATTGCCATTGGGTCTGCTGTGAGCAACGGGCAGAAGGACTTTGACACCATTGTGGCTGTCAGGCACCCCTACTCTGATGAG
56▶IThrValCysAlaGluAlal leAlal leGlySerAlaValSerAsnGlyGlnLysAspPheAspThrI leValAlaValArgHisProTyrSerAspGlu
 3601 GTGGACAGATCCATCAGGGTGGTCAGCCCTGTGGCATGTGCAGAGAGCTCATCTGACTATGCTCCTGACTGCTTTGTGCTCATTGAGATGAATGGCA
90▶ValAspArgSerI leArgValValSerProCysGlyMetCysArgGluLeul leSerAspTyrAlaProAspCysPheValLeul leGluMetAsnGlyL
 3701 AGCTGGTCAAAACCACCATTGAGGAATCATCCCCTCAAGTACACCAGGAACTAAACCTGAATTCGCTAGAGGGCCCTATTCTATAGTGTACCTAAAT
123▶ysLeuValLysThrThrI leGluGluLeul leProLeuLysTyrThrArgAsn•••
 3801 GCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCTCCCCCGTGCCTTCTTGACCCTGGAAGGTGCCACTC
 3901 CCACTGTCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGA

NotI (4047)

XhoI (4042)

4001 GGATTGGGAAGACAATAGCAGGCATGCGCAGGGCCCAATTGCTCGAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGT
 4101 GTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAAGTGCCAGAACATT
 4201 TCTCTATCGAA