

# pUNO1-hSTING-A162

Expression vector containing A162 isoform human STING (S162A) open reading frame

Catalog code: puno1-hsting-a162

<https://www.invivogen.com/hsting-a162>

For research use only

Version 19K10-MM

## PRODUCT INFORMATION

### Contents

- 20 µg of lyophilized plasmid DNA
- 2 x 1 ml blasticidin at 10 mg/ml

### Storage and Stability

- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20°C.
- Resuspended DNA should be stored at -20°C and is stable at least for 1 year.
- Store blasticidin at 4°C or -20°C. \*

\*The expiry date is specified on the product label.

### Quality control

- Plasmid construct has been confirmed by restriction analysis and full-length open reading frame (ORF) sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

## GENERAL PRODUCT USE

- **Subclone gene into another vector.** Two unique restriction sites flank the gene, allowing convenient excision. The 5' site is BspEI which is compatible with AgeI, XmaI, NgoMIV and SgrAI. The 3' site is NheI which is compatible with XbaI, SpeI, and AvrII.
- **Stable gene expression in mammalian cells.** pUNO1 plasmids can be used directly in transfection experiments both *in vitro* and *in vivo*. pUNO1 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*, as well as the selection of stable clones in mammalian cells using the same selective antibiotic. pUNO1 allows high levels of expression and secretion of the gene product.

## METHODS

### Plasmid resuspension

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

### Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT116 or other commonly used laboratory *E. coli* strains, such as DH5α.

### Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied as a 10 mg/ml colorless solution in HEPES buffer.

## PLASMID FEATURES

- **Bsr (blasticidin resistance gene):** The *bsr* gene from *Bacillus cereus* encodes a deaminase that confers resistance to the antibiotic blasticidin. The *bsr* gene is driven by the CMV promoter/enhancer in tandem with the bacterial EM7 promoter. Therefore, blasticidin can be used to select stable mammalian cells transfectants and *E. coli* transformants.
- **CMV promoter & enhancer** drives the expression of the blasticidin resistance in mammalian cells.

- **Human STING-A162**

**ORF size:** 1140 bp

**Cloning fragment size:** 1150 bp

STING (stimulator of interferon genes; also known as TMEM173, MITA, MPYS, and ERIS) is essential for the IFN response to microbial or self-DNA, and acts as a direct sensor of cyclic dinucleotides (CDNs). CDNs are important messengers in bacteria, affecting numerous responses of the prokaryotic cell, but also in mammalian cells, acting as agonists of the innate immune response. Human wild-type STING fails to bind DMXAA, a potent tumor vascular disrupting agent in mice<sup>1</sup>. The allele A162 contains a unique point mutation (S162A) placed at the cyclic-dinucleotide-binding site which confers DMXAA sensitivity to hSTING<sup>2</sup>.

- **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter<sup>3</sup> and the 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. It 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 Repeat<sup>4</sup> 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.
- **SV40 pAn:** The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions, resulting in high levels of steady-state mRNA<sup>5</sup>.
- **pMB1 ori** is a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.
- **Human beta-Globin polyA** is a strong polyadenylation (pAn) signal placed downstream of *bsr*. The use of beta-globin pAn minimizes interference<sup>6</sup> and possible recombination events with the SV40 polyadenylation signal.

1. Conlon J. *et al.*, 2013. Mouse, but not human STING, binds and signals in response to the vascular disrupting agent 5,6-dimethylxanthenone-4-acetic acid. *J Immunol* 190(10):5216-25. 2. Gao P. *et al.*, 2013. Structure-function analysis of STING activation by c[G(2',5')pA(3',5')p] and targeting by antiviral DMXAA. *Cell* 154(4):748-62. 3. Kim D. *et al.*, 1990. Use of the human elongation factor 1α promoter as a versatile and efficient expression system. *Gene* 91(2):217-23. 4. Takebe Y. *et al.*, 1988. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol Cell Biol* 8(1):466-72. 5. Carswell S. & Alwine J., 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol Cell Biol* 9(10):4248-58. 6. Yu J. & Russell J., 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human β-globin mRNA. *Mol Cell Biol* 21(17):5879-88.

## RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
ChemiComp GT116	Competent <i>E. coli</i>	gt116-11

### TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

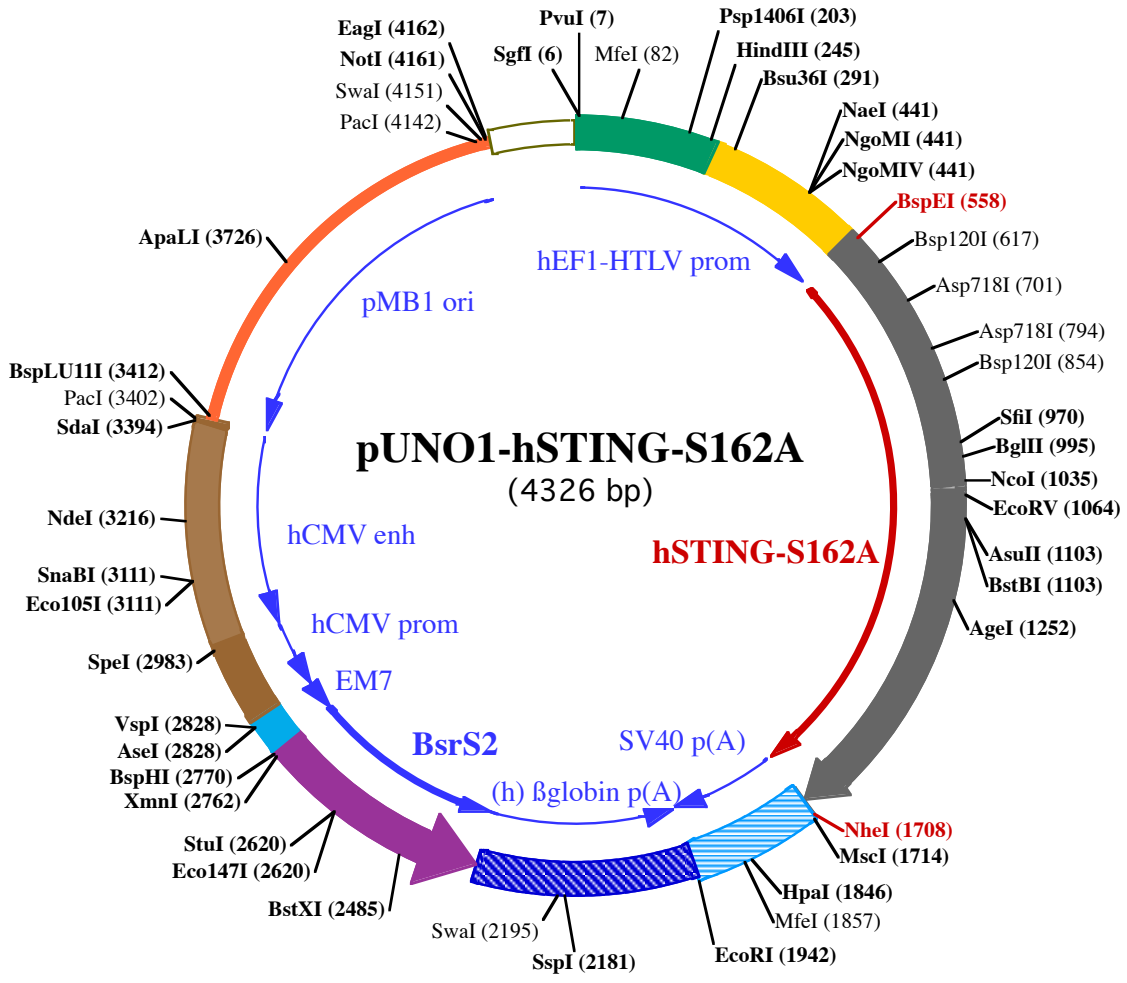
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InvivoGen Hong Kong: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)





**PvuI (7)**  
**SgfI (6)** 1 GGATCTGCGATCCGCTCCCGTCCCGTCACTGGGCGAGCGCACATCGCCACAGTCCCCGAGAAGTTGGGGGAGGGTCCGGCAATTGAACGGGTGCCTA

MfeI (82)  
101 GAGAAGGTGGCGGGGTAACGGGAAAGTATGCTGTACTGGCTCCGCCCTTTTCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCC

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

301 GCCATCCACGCCGGTTGAGTCGGCTTCTGCCGCTCCCGCTGTGGTGCCTCTGAAGTCCGCTCCGCTTCTAGGTAAGTTAAAGCTCAGGTCGAGACC

**NgoMIV (441)**  
**NgoMI (441)**  
**NaeI (441)**  
401 GGGCCTTTGTCGGCGCTCCCTTGAGGCTACCTAGACTCAGCCGGCTCTCCACGCTTGGCTGACCTGCTTCAACTCTACGCTTTGTTTCGTTT

**BspEI (558)**  
501 TCTGTTCTGCGCGTTACAGATCCAAGCTGTGACCGCCCTACCTGAGATCACGGCTCCGACAGCATGCCCACTCCAGCCTGCATCCATCCATCCC  
1 M P H S S L H P S I P

**Bsp120I (617)**  
601 GTGTCCAGGGTACGGGGCCAGAGGCGAGCCTTGGTCTGCTGAGTGCCTGCCTGGTACCCTTTGGGGCTAGGAGAGCCACCAGAGCACACTCTC  
11 C P R G H G A Q K A A L V L L S A C L V T L W G L G E P P E H T L

**Asp718I (701)** **Asp718I (794)**  
701 CGGTACCTGGTCTCCACCTAGCCTCCCTGCAGTGGGACTGCTGTTAAACGGGTCTGCAGCCTGGCTGAGGAGCTGCGCCACATCCACTCCAGGTACC  
45 R Y L V L H L A S L Q L G L L L N G V C S L A E E L R H I H S R Y

**Bsp120I (854)**  
801 GGGCAGCTACTGGAGGACTGTGCGGGCTGCCTGGGCTGCCCTCCGCGTGGGGCCTGTGCTGCTGCCATCTATTCTACTACTCCCTCCAAA  
78 R G S Y W R T V R A C L G C P L R R G A L L L L S I Y F Y Y S L P N

**SfiI (970)** **BglII (995)**  
901 TGGCTCGGCCCGCCCTCACTTGGATGCTTCCCTCCTGGCCTCTCGCAGGCACTGAACATCCTCCTGGCCTCAAGGGCTGGCCCCAGCTGAGATC  
111 A V G P P F T W M L A L L G L S Q A L N I L L G L K G L A P A E I

**NcoI (1035)** **EcoRV (1064)**  
1001 TCTGCAGTGTGTAAAAAGGAATTTCAACGTGGCCATGGGCTGGCATGGGCATATTACATCGGATATCTCGGGCTGATCCTGCCAGAGCTCCAGGCC  
145 S A V C E K G N F N V A H G L A W A Y Y I G Y L R L I L P E L Q A

**BstBI (1103)**  
**AsuII (1103)**  
1101 GGATTCGAACCTACAATCAGCATTACAACAACCTGCTACGGGTCAGTGAAGCCAGCGGCTGTATTTCTCTCCATTGGACTGTGGGGTGCCTGATAA  
178 R I R T Y N Q H Y N N L L R G A V S Q R L Y I L L P L D C G V P D N

**AgeI (1252)**  
1201 CCTGAGTATGGTACCCCAACATTCGTTCTGGATAAACTGCCCCAGCAGACCGGTGACCTGCTGGCATCAAGGATCGGGTTTACAGCAACAGCATC  
211 L S M A D P N I R F L D K L P Q Q T G D R A G I K D R V Y S N S I  
1301 TATGAGCTTCTGGAGAACGGGCGAGCGGGCAGCTGTGCTGAGTACGCCACCCCTTGCAGACTTTGTTGCCATGTCACAATACAGCTCAAGTG  
245 Y E L L E N G R A G T C V L E Y A T P L Q T L F A M S Q Y S Q A  
1401 GCTTTAGCCGGGAGGATAGGCTTGAAGCAGGCAAACTTCTGCGGACACTTGAAGACATCCTGGCAGATGCCCTGAGTCTCAGAACAGTCCGCGCT  
278 G F S R E D R L E Q A K L F C R T L E D I L A D A P E S Q N N C R L  
1501 CATTGCCTACCAGGAACCTGCAGATGACAGAGCTTCTCGCTGTCCAGGAGGTTCTCCGGCACCTGCGGCAGGAGGAAAAGGAAAGAGTTACTGTGGGC  
311 I A Y Q E P A D D S S F S L S Q E V L R H L R Q E E K E E V T V G  
1601 AGCTTGAAGACTCAGCGGTGCCAGTACCTCCAGATGTCCAAAGCCTGAGCTCCTCATGGAATGGAAAAGCCCTCCCTCCCGCAGCGGAT  
345 S L K T S A V P S T S T M S Q E P E L L I S G M E K P L P L R T D

**MscI (1714)**  
**NheI (1708)**  
1701 TCTCTTGTAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTGGACAAACCACTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTG  
378 F S •

**HpaI (1846)** **MfeI (1857)**  
1801 TGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAAGTTAAACAACAATTGCATTCAATTTATGTTTCAGGTTCCAGGGGAGGTGTGG

**EcoRI (1942)**  
1901 GAGGTTTTTAAAGCAAGTAAACCTCTACAATGTGGTATGGAATTTAAAATACAGCATAGCAAACTTTAACCTCCAATCAAGCCTCTACTTGAAT  
2001 CCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGCTGTGGCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTAAGATATAG

**SspI (2181)** **SwaI (2195)**  
2101 TGTATTTTCCCAAGGTTTGAAGTACTCTTCATTTCTTTATGTTTTAAATGCAGTACCTCCACATCCCTTTTGTAGAAAATATTCAGAAAATATTTA  
2201 AATACATATTGCAATGAAAATAAATGTTTTTATTAGGCAGAAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGA  
2301 ACAAAGAACCTTTAATAGAAATTTGACAGCAAGAAAGCAGCTTCTAGCTTTAGTTCTGCTGACTTGGGGGATGAGTTCCTCAATGGTGGTTTTG  
144 • N R T Y K L P I L E E I T T K

**BstXI (2485)**  
2401 ACCAGCTTGCATTCATCTCAATGAGCACAAGCAGTCAAGGATAGTCAAGATGAGCTCTGACATGCCAGGGGCTGACCCCTGATGGATC  
124 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 V V R I S R  
2501 TGTCCACCTCATCAGAGTAGGGTGCCTGACAGCCCAATGGTCAAAGTCTTCTGCCGTTGCTCAGCAGACCAATGGCAATGGCTTCAGCACA  
91 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 A I A E A C

**StuI (2620)**  
**Eco147I (2620)**  
2601 GACAGTGACCCTGCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCAAGTCTTGGTCTGATGGCCGCCCCGACATGGTCTTGTGCTCTCA  
58 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 H K N D E

**BspHI (2770)**  
**XmnI (2762)**  
2701 TAGAGCATGGTATCTTCTCAGTGGCGACCTCCACAGCTCCAGATCTGCTGAGAGATGTTGAAGGTCTTCAATGATGGCCCTCTATAGTGAAGTGTAT  
24 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 (2828)**  
**AseI (2828)**

2801 TATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCGCTTATAT

**SpeI (2983)**

2901 AGACCTCCACCGTACACGCCTACCGCCATTTCGTCGAATGGGCGGAGTTGTTACGACATTTTGAAAGTCCCGTTGATTTACTAGTCAAACAAACT

3001 CCCATTGACGTCATGGGTTGGAGACTTGAAATCCCGTGAGTCAAACCGCTATCCACGCCATTGATGACTGCCAAAACCGCATCATCATGGTAATA

**SnaBI (3111)**  
**Eco105I (3111)**

3101 GCGATGACTAATACGTAGATGACTGCCAAGTAGGAAAGTCCATAAGGTCATGACTGGGCATAATGCCAGGCGGGCCATTACCGTCATTGACGTCAA

**NdeI (3216)**

3201 TAGGGGGCTACTTGGCATATGATACACTTGATGACTGCCAAGTGGGCGAGTTACCGTAAATACTCCACCCATTGACGTCATGGAAAGTCCCTATTGG

**SdaI (3394)**

3301 CGTTACTATGGGAACATACGTCATTATTGACGTCATGGGCGGGGTCGTTGGGCGGTGAGCCAGGCGGGCCATTACCGTAAGTTATGTAACGCCTGCA

**PacI (3402)** **BspLU11I (3412)**

3401 GGTTAATTAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGCCCGCTTGCTGGCGTTTTTCCATAGGCTCCGCCCTGACGA

3501 GCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTCCCTGGAAGTCCCTCGTGGCTCTCCT

3601 GTTCCGACCTGCCGTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGT

**ApaLI (3726)**

3701 AGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCGACCGTGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGTAAG

3801 ACACGACTTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTAC

3901 GGTACTACTAGAAGACAGTATTTGGTATCTGCGCTGTGTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTTTGATCCGGCAAACAAACCACCG

4001 CTGGTAGCGGTGTTTTTTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCA

**EagI (4162)**

**PacI (4142)** **SwaI (4151)** **NotI (4161)**

4101 GTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAAATATCTTTATTTTCATTACATCTGT

4201 GTGTTGGTTTTTTGTGTAATCGTAACTAACATACGCTCTCCATCAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCAGTGAAGTGC

4301 AGGTGCCAGAACATTTCTCTATCGAA