pUNO1-hSTING-HAQ

Expression vector containing HAQ isoform human STING (R71H-G230A-R293Q) open reading frame

Catalog code: puno1-hsting-haq

https://www.invivogen.com/hsting-haq

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 at 10 mg/ml 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-HAQ**

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. Several non-synonymous variants of STING have been described in the human population. STING-HAQ has been identified as a common haplotype (~20% of the human population and found in THP1 cells). HAQ contains three non-synonymous single nucleotide substitutions; R71H, G230A and R293Q. STING-HAQ expresses a STING protein that displays a reduced intrinsic IFN-β stimulatory activity[1,2] but retains the ability to respond to metazoan and bacterial CDNs[2].

• **EF-1α/HTLV hybrid promoter** is a composite promoter comprised of the Elongation Factor-1α (EF-1α) core promoter[3] 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[4] 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[5].

• **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[6] and possible recombination events with the SV40 polyadenylation signal.

1. Jin L. *et al.*, 2011. Identification and characterization of a loss-of-function human MPYS variant. Genes Immun. 12(4):263-9. **2. Yi G. *et al.*, 2013.** Single nucleotide polymorphisms of human STING can affect Innate immune response to cyclic dinucleotides. PLoS One 8(10):e77846. **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 *E. coli*	gt116-11

TECHNICAL SUPPORT
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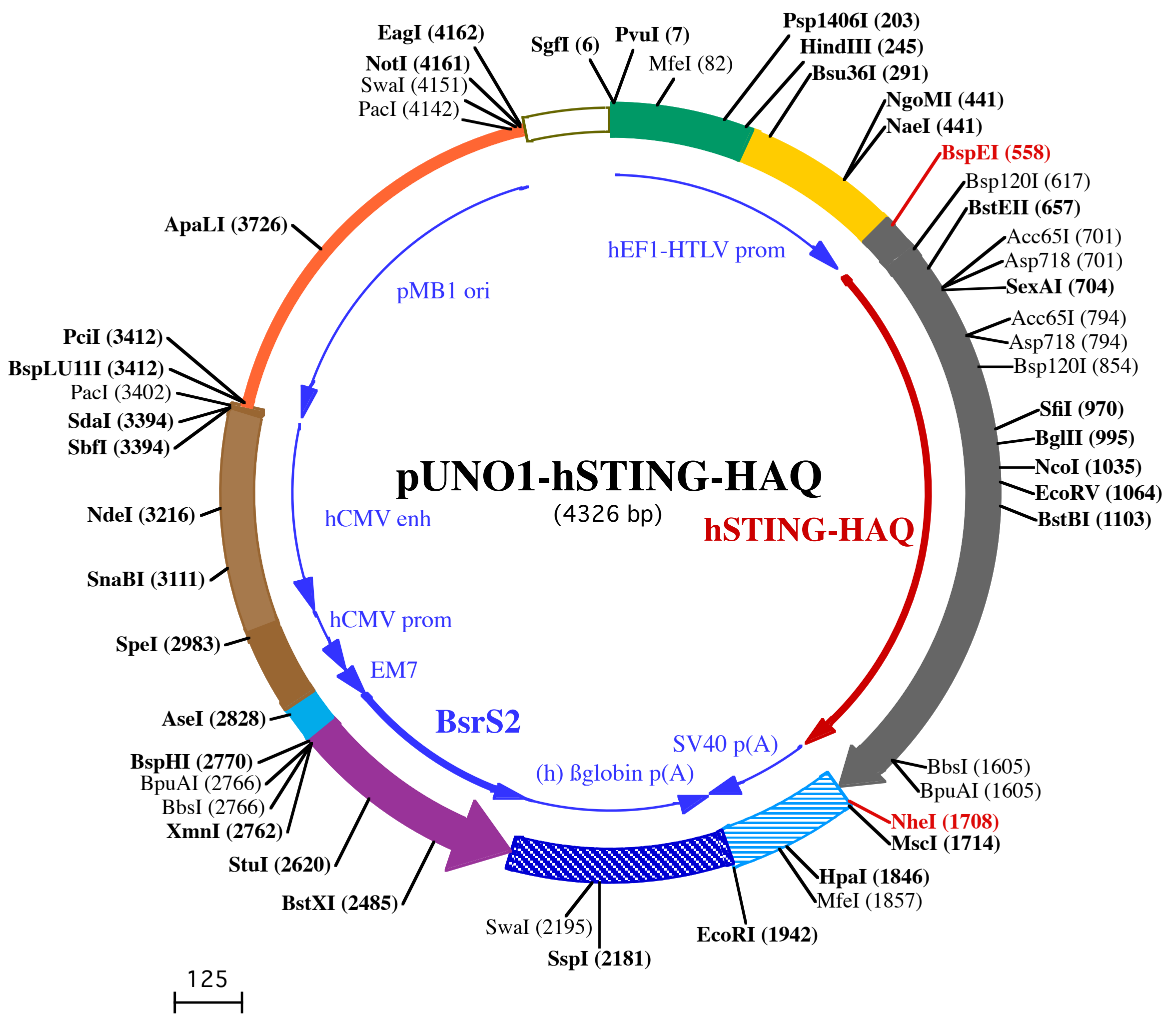
pUNO1-hSTING-HAQ
(4326 bp)
hSTING-HAQ
BsrS2
hEF1-HTLV prom
pMB1 ori
hCMV enh
hCMV prom
EM7
SV40 p(A)
(h) ßglobin p(A)
SgfI (6)
PvuI (7)
MfeI (82)
Psp1406I (203)
HindIII (245)
Bsu36I (291)
NgoMI (441)
NaeI (441)
BspEI (558)
Bsp120I (617)
BstEII (657)
Acc65I (701)
Asp718 (701)
SexAI (704)
Acc65I (794)
Asp718 (794)
Bsp120I (854)
SfiI (970)
BglII (995)
NcoI (1035)
EcoRV (1064)
BstBI (1103)
BbsI (1605)
BpuAI (1605)
NheI (1708)
MscI (1714)
HpaI (1846)
MfeI (1857)
EcoRI (1942)
SwaI (2195)
SspI (2181)
BstXI (2485)
StuI (2620)
XmnI (2762)
BbsI (2766)
BpuAI (2766)
BspHI (2770)
AseI (2828)
SpeI (2983)
SnaBI (3111)
NdeI (3216)
SbfI (3394)
SdaI (3394)
PacI (3402)
BspLU11I (3412)
PciI (3412)
ApaLI (3726)
PacI (4142)
SwaI (4151)
NotI (4161)
EagI (4162)
125

PvuI (7)
SgfI (6) MfeI (82)
1 GGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACGGGTGCCTA

101 GAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCC

Psp1406I (203) HindIII (245) Bsu36I (291)
201 GTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCC

301 GCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACC

NgoMI (441)
NaeI (441)
401 GGGCCTTTGTCCGGCGCTCCCTTGGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTT

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

Bsp120I (617) BstEII (657)
601 GTGTCCCAGGGGTCACGGGGCCCAGAAGGCAGCCTTGGTTCTGCTGAGTGCCTGCCTGGTGACCCTTTGGGGGCTAGGAGAGCCACCAGAGCACACTCTC
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

SexAI (704)
Asp718 (701) Asp718 (794)
Acc65I (701) Acc65I (794)
701 CGGTACCTGGTGCTCCACCTAGCCTCCCTGCAGCTGGGACTGCTGTTAAACGGGGTCTGCAGCCTGGCTGAGGAGCTGCACCACATCCACTCCAGGTACC
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 H H I H S R Y

Bsp120I (854)
801 GGGGCAGCTACTGGAGGACTGTGCGGGCCTGCCTGGGCTGCCCCCTCCGCCGTGGGGCCCTGTTGCTGCTGTCCATCTATTTCTACTACTCCCTCCCAAA
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 TGCGGTCGGCCCGCCCTTCACTTGGATGCTTGCCCTCCTGGGCCTCTCGCAGGCACTGAACATCCTCCTGGGCCTCAAGGGCCTGGCCCCAGCTGAGATC
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 TCTGCAGTGTGTGAAAAAGGGAATTTCAACGTGGCCCATGGGCTGGCATGGTCATATTACATCGGATATCTGCGGCTGATCCTGCCAGAGCTCCAGGCCC
145 S A V C E K G N F N V A H G L A W S Y Y I G Y L R L I L P E L Q A

BstBI (1103)
1101 GGATTCGAACTTACAATCAGCATTACAACAACCTGCTACGGGGTGCAGTGAGCCAGCGGCTGTATATTCTCCTCCCATTGGACTGTGGGGTGCCTGATAA
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
1201 CCTGAGTATGGCTGACCCCAACATTCGCTTCCTGGATAAACTGCCCCAGCAGACCGCTGACCGTGCTGGCATCAAGGATCGGGTTTACAGCAACAGCATC
211 L S M A D P N I R F L D K L P Q Q T A D R A G I K D R V Y S N S I
1301 TATGAGCTTCTGGAGAACGGGCAGCGGGCGGGCACCTGTGTCCTGGAGTACGCCACCCCCTTGCAGACTTTGTTTGCCATGTCACAATACAGTCAAGCTG
245 Y E L L E N G Q 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 GCTTTAGCCGGGAGGATAGGCTTGAGCAGGCCAAACTCTTCTGCCAGACACTTGAGGACATCCTGGCAGATGCCCCTGAGTCTCAGAACAACTGCCGCCT
278 G F S R E D R L E Q A K L F C Q T L E D I L A D A P E S Q N N C R L
1501 CATTGCCTACCAGGAACCTGCAGATGACAGCAGCTTCTCGCTGTCCCAGGAGGTTCTCCGGCACCTGCGGCAGGAGGAAAAGGAAGAGGTTACTGTGGGC
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

BpuAI (1605)
BbsI (1605)
1601 AGCTTGAAGACCTCAGCGGTGCCCAGTACCTCCACGATGTCCCAAGAGCCTGAGCTCCTCATCAGTGGAATGGAAAAGCCCCTCCCTCTCCGCACGGATT
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 TCTCTTGAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTG
378 F S •

HpaI (1846) MfeI (1857)
1801 TGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGG

EcoRI (1942)
1901 GAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAAT

2001 CCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAG

SspI (2181) SwaI (2195)
2101 TGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTA

2201 AATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGA

2301 ACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCCTGGTGTACTTGAGGGGGATGAGTTCCTCAATGGTGGTTTTG
141 • N R T Y K L P I L E E I T T K

BstXI (2485)
2401 ACCAGCTTGCCATTCATCTCAATGAGCACAAAGCAGTCAGGAGCATAGTCAGAGATGAGCTCTCTGCACATGCCACAGGGGCTGACCACCCTGATGGATC
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 TGTCCACCTCATCAGAGTAGGGGTGCCTGACAGCCACAATGGTGTCAAAGTCCTTCTGCCCGTTGCTCACAGCAGACCCAATGGCAATGGCTTCAGCACA
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)
2601 GACAGTGACCCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCCCAGTCTTGGTCCTGATGGCCGCCCCGACATGGTGCTTGTTGTCCTCA
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)
BpuAI (2766)
BbsI (2766)
XmnI (2762)

2701 TAGAGCATGGTGATCTTCTCAGTGGCGACCTCCACCAGCTCCAGATCCTGCTGAGAGATGTTGAAGGTCTTCATGATGGCCCTCCTATAGTGAGTCGTAT
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

AseI (2828)

2801 TATACTATGCCGATATACTATGCCGATGATTAATTGTCAAAACAGCGTGGATGGCGTCTCCAGCTTATCTGACGGTTCACTAAACGAGCTCTGCTTATAT

SpeI (2983)

2901 AGACCTCCCACCGTACACGCCTACCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAACAAACT

3001 CCCATTGACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCCATTGATGTACTGCCAAAACCGCATCATCATGGTAATA

SnaBI (3111)

3101 GCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAA

NdeI (3216)

3201 TAGGGGGCGTACTTGGCATATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGG

SdaI (3394)
SbfI (3394)

3301 CGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCGTTGGGCGGTCAGCCAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTGCA

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

3401 GGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGA

3501 GCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCT

3601 GTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGT

ApaLI (3726)

3701 AGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAG

3801 ACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTAC

3901 GGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCG

4001 CTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCA

EagI (4162)
PacI (4142) SwaI (4151) **NotI (4161)**

4101 GTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCAGCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGT

4201 GTGTTGGTTTTTTGTGTGAATCGTAACTAACATACGCTCTCCATCAAAACAAAACGAAACAAAACAAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGC
4301 AGGTGCCAGAACATTTCTCTATCGAA