

STOP

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TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com



pCpGfree-OVA

An OVA-expressing DNA immunization plasmid completely devoid of CpG dinucleotides

Catalog # pcpgf-ova

For research use only

Version # 21F04-MMv02

PRODUCT INFORMATION

Content:

- 20 µg of pCpGfree-OVA plasmid provided as lyophilized DNA
- *E. coli* GT115 strain provided lyophilized on a paper disk
- 1 ml of Zeocin™ (100 mg/ml)

Storage and Stability:

- Products are shipped at room temperature.
- Lyophilized DNA is stable when stored at -20 °C.
- Resuspended DNA is stable for 12 months when stored at -20 °C.
- Bacteria should be stored at -20 °C and are stable for at least 1 year.
- Store Zeocin™ at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control:

Plasmid construct has been confirmed by restriction analysis and sequencing. Plasmid DNA was purified by ion exchange chromatography and lyophilized. Viability of the lyophilized bacteria upon resuspension has been verified.

GENERAL PRODUCT USE

InvivoGen has developed a family of plasmids that are completely devoid of CpG dinucleotides. These plasmids yield high levels of transgene expression both *in vitro* and *in vivo*, and in contrast to CMV-based plasmids allow sustained expression *in vivo*.

pCpGfree plasmids contain elements that naturally lack CpG dinucleotides, were modified to remove all CpGs, or entirely synthesized such as genes encoding selectable markers or reporters.

pCpGfree-OVA expresses a CpG-free allele of the ovalbumin (OVA) gene. This plasmid is designed for DNA immunization experiments in animal models. This plasmid possesses dual functions; it can be used as a DNA vaccine carrier for antigen presentation, and as an immune-stimulative adjuvant¹.

PLASMID FEATURES

All the elements required for replication and selection of the plasmid in *E. coli* and gene expression in mammalian cells are completely devoid of CpG dinucleotides. Furthermore, all Dam methylation sites (GATC) have been removed to prevent prokaryotic methylation.

Elements for expression in *E. coli*

- Origin of replication: The *E. coli* R6K gamma ori has been modified to remove all CpGs. This origin is activated by the R6K specific initiator protein π , encoded by the *pir* gene².
- Bacterial promoter: EM2K is a CpG-free version of the bacterial EM7 promoter.
- Selectable marker: The Zeocin™ resistance gene is a small gene (<400 bp) that contains numerous CpG dinucleotides. A synthetic new allele was created that contains no CpGs.

Elements for expression in mammalian cells

- Mammalian promoter: The CpG-free promoter combines the mouse CMV enhancer, the human elongation factor 1 alpha core promoter and 5'UTR containing a synthetic intron.
- Polyadenylation signal: The polyadenylation signal is a CpG-free form of the late SV40 polyadenylation signal.

- MAR: Matrix attached regions (MARs) are sequences typically AT-rich that are able to form barriers between independently regulated domains³. pCpG plasmids contains two MARs, from the 5' region of the human IFN- β gene or β -globin gene that were chosen because they are naturally CpG-free. The MARs are placed between the bacterial and mammalian transcription units.

- pCpG-OVA expresses a synthetic OVA gene, a CpG-free allele of the ovalbumin (OVA) gene constructed by chemical synthesis.

Due to the presence of the R6K γ origin of replication, pCpG plasmids can only be amplified in *E. coli* mutant strain expressing a *pir* mutant gene. They will not replicate in standard *E. coli* strains. Therefore, pCpG plasmids are provided with the *E. coli* GT115 strain, a *pir* mutant also deficient in *Dcm* methylation.

1. Miura N. et al., 2015. A KALA-modified lipid nanoparticle containing CpG-free plasmid DNA as a potential DNA vaccine carrier for antigen presentation and as an immune-stimulative adjuvant. *Nucleic Acids Res.* 43(3):1317-31. 2. Wu F. et al. 1995. A DNA segment conferring stable maintenance on R6K gamma-origin core replicons. *J Bacteriol.* 177(22):6338-45. 3. Bode J. et al., 1996. Scaffold/matrix-attached regions: topological switches with multiple regulatory functions. *Crit Rev Eukaryot Gene Expr.* 6(2-3):115-38.

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 H₂O. Store resuspended plasmid at -20 °C.

Reconstitution of *E. coli* GT115 strain

Use sterile conditions to do the following:

1. Reconstitute *E. coli* GT115 by adding 1 ml of Luria-Bertani (LB) medium in the tube containing the paper disk. Let sit for 5 minutes.
2. Mix gently by vortexing for 1-2 minutes.
3. Streak bacteria taken from this suspension on a LB agar plate.
4. Place the plate in an incubator at 37 °C overnight.
5. Isolate a single colony and grow the bacteria in LB or terrific broth (TB) medium.
6. Prepare competent cells utilizing protocol of choice.

Plasmid amplification and cloning

Plasmid amplification and cloning can be performed in *E. coli* GT115.

Zeocin™ usage

This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media.

TECHNICAL SUPPORT

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

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InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3-622-34-80

E-mail: info@invivogen.com

RELATED PRODUCTS

Product	Description	Catalog Code
ChemiComp GT115	Chemically competent <i>E. coli</i>	gt115-11
pCpGfree-mcs	CpG-free cloning vector	pcpgf-mcs
Zeocin™	Selective antibiotic for the <i>Sh ble</i> gene	ant-zn-1
OVA Peptides Ova		
257-264	For detection; ELISPOT	vac-sin
Ova 323-339	For detection; ELISPOT	vac-isq

TECHNICAL SUPPORT

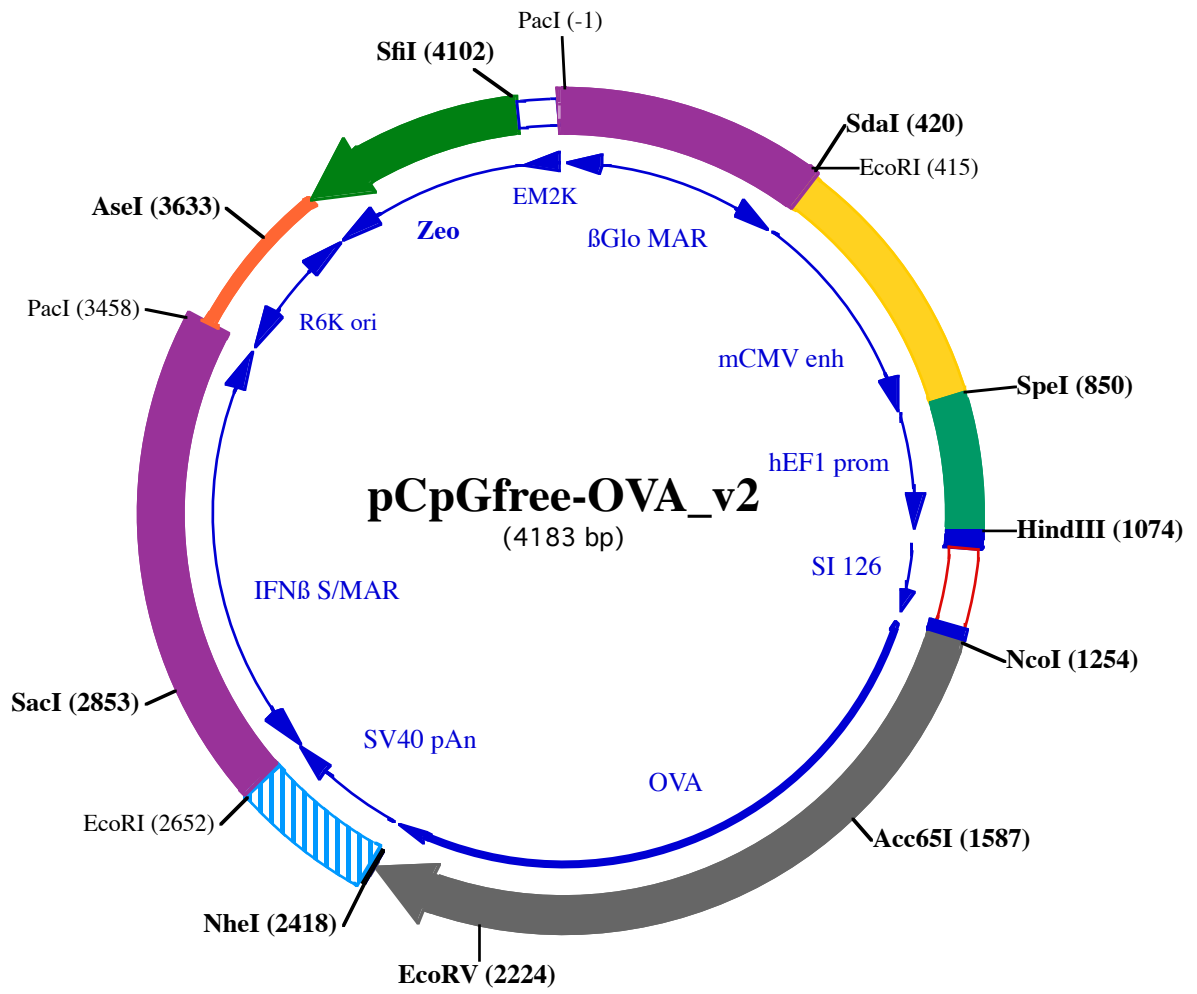
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1 Pacl (-1)
1 TTAATTAATAATTATCTCTAAGGCATGTGAAGCTGGCTGCTTGGTTTTTCATCTGTACTTCTCATCTGCTACCTCTGTGACCTGAAACATATTTATAATTCCAT
101 TAAGCTGTGCATATGATAGATTTATCATATGATTTTTCTTAAAGGATTTTTGTAAGAACTAATTGAATTGATACCTGTAAGTCTTTATCACACTACCC
201 AATAAATAATAAATCTCTTTGTTGAGCTCTCTGTTTCTATAAATATGTACCAGTTTTATTGTTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATATAT
301 ACACACACATGTGTGCATTATAAATATATAACAATTTTTATGAATAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTTATGTGAGCAA

SdaI (420)
EcoRI (415)
401 ACAGCAGATTAAAGGAATTCCTGCAGGAGTCAATGGGAAAAACCCATTGGAGCCAAGTACACTGACTCAATAGGGACTTTCCATTGGGTTTTGCCAGT
501 ACATAAGGTCAATAGGGGGTGAAGTCAACAGGAAAGTCCCATTGGAGCCAAGTACATTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAG
601 GTCAATGGGAGGTAAAGCAATGGGTTTTTCCCATTACTGACATGATACTGAGTCAATAGGGACTTTCCAATGGGTTTTGCCAGTACATAAGGTCAATA
701 GGGGTGAATCAACAGGAAAGTCCCATTGGAGCCAAGTACACTGAGTCAATAGGGACTTTCCATTGGGTTTTGCCAGTACAAAAGGTCAATAGGGGGTGA

SpeI (850)
801 GTCAATGGGTTTTTCCCATTATTGGCACATACATAAGGTCAATAGGGGTGACTAGTGGAGAAGAGCATGCTTGAGGGCTGAGTCCCTCAGTGGGCAGA
901 GAGCACATGGCCACAGTCCCTGAGAAGTTGGGGGAGGGTGGGCAATTGAACTGGTGCCTAGAGAAGGTGGGCTGGGTAAGTGGGAAAGTATGATG

HindIII (1074)
1001 GGTGACTGGCTCCACCTTTTTCCCAGGGTGGGGGAGAACCATATATAAGTGCAGTAGTCTGTGAACATTCAAGCTTCTGCCTTCTCCCTCCTGTGA
1101 GTTTGtaagtcactgactgtctatgctgggaaagggggcaggaggtggggcagtcaggaaaagtggcactgtgaacctgcagccctagacaatt

NeoI (1254)
1201 gtactaaccttcttctcttctctctctgacagGTTGGTGTACAGTAGTCCACCATGGGCTCAATTGGTGCAGCATCAATGGAGTCTGCTTTGATGT
1301 TTTCAAGGAGCTGAAAGTGCACCATGCAATGAGAATATTTTTACTGCCAATAGCAATAATGTCAGCCCTTGCATGGTGTATCTGGGGCCAAGGAC
15▶ F K E L K V H H A N E N I F Y C P I A I M S A L A M V Y L G A K D
1401 TCCACCAGAACCCAAATCAACAAGGTTGTAAGTTTTGACAAGCTGCCAGGCTTTGGTACTCAATAGAGGCCAGTGTGGCACCAGTGTAAATGTACACT
49▶ S T R T Q I N K V V R F D K L P G F G D S I E A Q C G T S V N V H

Acc65I (1587)
1501 CCTCCCTAAGGGATATACTGAACCAGATAACCAAGCCCAATGATGTGACAGCTTCTCCTTGGCAAGCAGACTATATGCAGAGGAGAGGTACCCAATCTT
82▶ S S L R D I L N Q I T K P N D V Y S F S L A S R L Y A E E R Y P I L
1601 GCCTGAATACCTGCAGTGTGTCAAGGAATTTACAGAGGGGGCTAGAGCCCATCAACTTTCAGACTGCAGCTGACCAAGCAAGGGAGTTAATCAACTCT
115▶ P E Y L Q C V K E L Y R G G L E P I N F Q T A A D Q A R E L I N S
1701 TGGGTGGAGAGCCAGACCAATGGAATAATCAGGAATGTTCTGAGCCTTCTCATCTGACTCCAGACAGCAATGGTCTTGGTCAATGCAATTGTCTTCA
149▶ W V E S Q T N G I I R N V L Q P S S V D S Q T A M V L V N A I V F
1801 AGGGCTGTGGGAGAAGCTTTCAAAGATGAAGACACTCAGGCAATGCCCTCAGAGTAACTGAACAGGAGTCCAAACCTGTGCAGATGATGTACCAAT
182▶ K G L W E K T F K D E D T Q A M P F R V T E Q E S K P V Q M M Y Q I
1901 TGGGTTATTGAGGGTGGCTTCAATGGCTTCTGAGAAAATGAAGATTCTGGAGTTACCCTTGGCAGTGGGACAATGTCTATGCTGGTCTGTACCAGAT
215▶ G L F R V A S M A S E K M K I L E L P F A S G T M S M L V L L P D
2001 GAGGTGTGAGGGCTGAGCAGCTGGAGTCAATCATCAATTTTGAAGATTAACAGAGTGGACCTCCTCAATGTGATGGAAGAAAGGAAAATCAAGGTCT
249▶ E V S G L E Q L E S I I N F E K L T E W T S S N V M E E R K I K V
2101 ACCTGCCAGAATGAAAATGGAGGAGAAAATACAACCTCACCTCAGTGTGATGGCAATGGGATAACAGATGTCTTCTCCAGCTGTGCCAACCTCTCTGG
282▶ Y L P R M K M E E K Y N L T S V L M A M G I T D V F S S S A N L S G

EcoRV (2224)
2201 CATCAGCAGTGTGAATCCCTAAAGATATCACAGGCTGTTTATGAGCCCATGCAGAAATCAATGAAGCAGGCAGGGAGGTGGTGGGCTCTGCTGAGGCA
315▶ I S S A E S L K I S Q A V H A A H A E I N E A G R E V V G S A E A
2301 GGAGTGGATGCTGCCTCTGCTCAGAAGAGTTGAGAGCAGACCACCCCTTCTCTTCTGCATCAAGCATATAGCCACCAATGCTGTTCTTTCTTTGGAA
349▶ G V D A A S V S E E F R A D H P F L F C I K H I A T N A V L F F G

NheI (2418)
2401 GGTGTGTGCCCCCTAAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAATAAATGCTTTATTT
382▶ R C V S P •
2501 GTGAAATTTGTGATGCTATTGCTTTATTTGTAACATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCAATTTATGTTTCAGGTTGAGG

EcoRI (2652)
2601 GGAGGTGTGGGAGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTCAGTCAATATGTTACCCCAAAAAGCTGTTGTTAACTTGC
2701 AACCTCATTCTAAAATGTATATAGAAGCCAAAAGACAATAACAAAATATTCTTGTAGAACAAAATGGGAAAGAATGTCCACTAAATATCAAGATTTA

SacI (2853)
2801 GAGCAAAGCATGAGATGTGTGGGATAGACAGTGAAGGCTGATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTACCTATGAAAA

2901 AATATGGCATTTTACAATGGGAAATGATGGTCTTTTTCTTTTTAGAAAAACAGGGAATATATTTATATGTAAAAATAAAAGGGAACCCATATGTCA
3001 TACCATACACACAAAAAATCCAGTGAATTATAAGTCTAAATGGAGAAGGCAAACTTTAAATCTTTAGAAAATAATATAGAAGCATGCCATCAAGAC
3101 TTCAGTGTAGAGAAAAATTTCTTATGACTCAAAGTCTAACCCACAAAGAAAAGATTGTTAATTAGATTGCATGAATATTAAGACTTATTTTTAAATTA
3201 AAAACCATTAAGAAAAGTCAGGCCATAGAATGACAGAAAATATTTGCAACACCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAAGTCTTACA
3301 AATCAGTAAAAATAAACTAGACAAAAATTTGAACAGATGAAAGAGAACTCTAAATAATCATTACACATGAGAACTCAATCTCAGAAATCAGAGAAC

PacI (3458)

3401 TATCATTGCATATACACTAAATTAGAGAAATATTTAAAAGGCTAAGTAACATCTGTGGCTTAATTTAAATCAGCAGTTCAACCTGTTGATAGTATGACTA
3501 AGCTCTCATGTTAATGTACTAAGCTCTCATGTTAATGAACTAAACCCTCATGGCTAATGTACTAAGCTCTCATGGCTAATGTACTAAGCTCTCATGTT

AseI (3633)

3601 TCATGTAATAAGCTCTCATGTTTGAACAATAAAATTAATATAAATCAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAGAATATA

3701 TAAGGCTTTTAAAGGTTTAAAGGTTTCTAGCTTGTAGTCTGTTCTCAGCTACAAAATGGACACAATTTCCAGCAGGGTCTCTGAGGGCAAATTCCTT

125 • D Q E E A V F H V C N G A P D R L A F E R

3801 CCCCAAGTTGTTACCAATTTCTGTCATGGCTGGGCCAGAGGCATCCCTGAAATTTGTGCTGACTACTTCTGACCATTCTGCATAAAGCTCATCTAGGC

102 G W P Q E G I E T M A P G S A D R F N T S V V E S W E A Y L E D L G

3901 CTCTGACCCAGACCAAGCAAGGGTGTGTCAGGGACAACCTGGTCTGAACTGCTGAGATGAAGAGGGTGACATCATCTCTGACAACACCAGCAAAATC

69 R V W V W A L T N D P V V Q D Q V A S I F L T V D D R V V G A F D

4001 ATCTTCAACAAAGTCTCTGGAGAATCCTAATCTGTCAGTCCAGAACTCTACAGCCCCTGCAACATCCCTTGCTGTGAGGACTGGGACTGCAGAAGTGAGT

36 D E V F D R S F G L R D T W F E V A G A V D R A T L V P V A S T L

SfiI (4102)

4101 TTGGCCATGATGGCCCTCCTATAGTGAGTTGATTATACTATGCAGATATACTATGCCAATGTTAATTGTCAACTACCTGTT

2 K A M