

# 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](mailto:info@invivogen.com)



# pCpGfree-OVA

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

Catalog # pcpgf-ova

For research use only

Version # 16C09-MMv02

## PRODUCT INFORMATION

### Content:

- 20 µg of pCpGfree-OVA plasmid provided as lyophilized DNA
- *E. coli* GT115 strain provided lyophilized on a paper disk
- 4 pouches of Fast-Media® Zeo (2 TB and 2 Agar)

### 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 Fast-Media® Zeo at room temperature. Fast-Media® pouches are stable for 18 months when stored properly.

### 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 adjuvant<sup>1</sup>.

## 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  $\pi$ , encoded by the *pir* gene<sup>2</sup>.
- 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 domains<sup>3</sup>. pCpG plasmids contains two MARs, from the 5' region of the human IFN- $\beta$  gene or  $\beta$ -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 $\gamma$  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 H<sub>2</sub>O. 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 LB medium in the tube containing the paper disk. Let sit for 5 minutes. Mix gently by vortexing for 1-2 minutes.
- 2- Streak bacteria taken from this suspension on a LB agar plate.
- 3- Place the plate in an incubator at 37°C overnight.
- 4- Isolate a single colony and grow the bacteria in LB or TB medium.
- 5- Prepare competent cells utilizing protocol of choice.

### Preparation of Fast-Media®

pCpGfree-OVA is provided with 4 pouches of Fast-Media® Zeo (2 TB and 2 Agar) to facilitate the preparation of liquid and solid zeocin-selection media by using a microwave.

- 1- Pour the contents of a Fast-Media® 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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E-mail: info@invivogen.com

## RELATED PRODUCTS

Product	Description	Catalog Code
ChemiComp GT115	Chemically competent <i>E. coli</i>	gt115-11
Fast-Media® Zeo Agar	Solid agar selection medium - Zeocin™	fas-zn-s
Fast-Media® Zeo TB	Liquid TB selection medium - Zeocin™	fas-zn-l
pCpGfree-mcs	CpG-free cloning vector	pcpgf-mcs
Zeocin™	Selective antibiotic for the <i>Sh ble</i> gene	ant-zn-1
<b>OVA Peptides</b>		
Ova 257-264	For detection; ELISPOT	vac-sin
Ova 323-339	For detection; ELISPOT	vac-isq

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### TECHNICAL SUPPORT

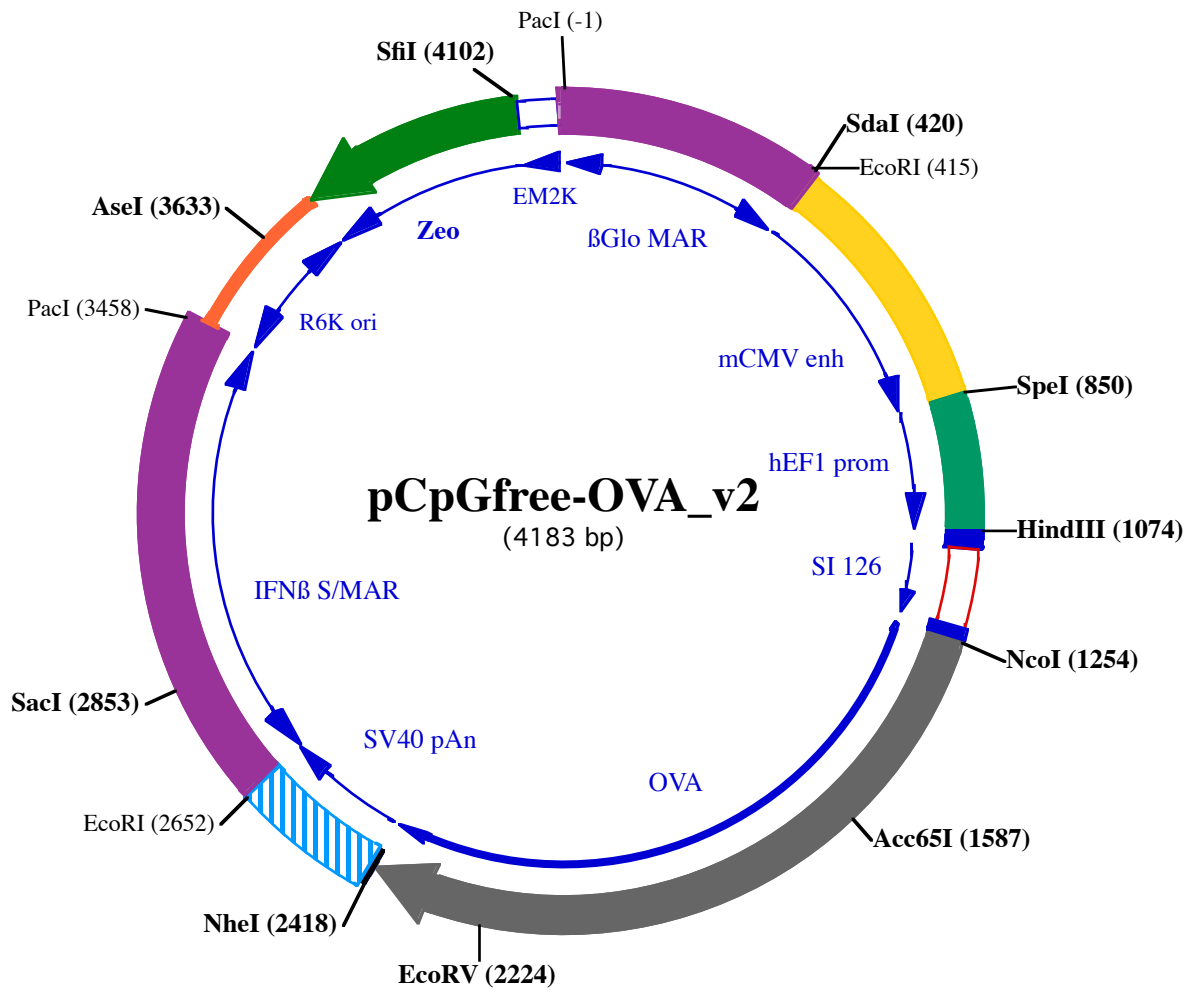
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E-mail: [info@invivogen.com](mailto:info@invivogen.com)



125  
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1 Pacl (-1)  
1 TTAATTAATAAATTATCTCTAAGGCATGTGAACTGGCTGCTTGGTTTTTCATCTGTACTTCTATCTGCTACCTCTGTGACCTGAAACATATTTATAATTCCAT  
101 TAAGCTGTGCATATGATAGATTTATCATATGATTTTTCTTAAAGGATTTTTGTAAGAACTAATTGAATTGATACCTGTAAGTCTTTATCACACTACCC  
201 AATAAATAATAAATCTCTTTGTTGAGCTCTCTGTTTCTATAAATATGTACCAGTTTTATTGTTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATATAT  
301 ACACACACATGTGTGCATTACATAAATATATAACAATTTTTATGAATAAAAAAATTATTAGCAATCAATATTGAAAACCACTGATTTTTGTTTTATGTGAGCAA

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

SpeI (850)  
801 GTCAATGGGTTTTTCCCATTATTGGCACATACATAAGGTCAATAGGGGTGACTAGTGGAGAAGAGCATGCTTGAGGGCTGAGTCCCTCAGTGGGCAGA  
901 GAGCACATGGCCACAGTCCCTGAGAAGTTGGGGGAGGGTGGGCAATTGAACTGGTGCCTAGAGAAGGTGGGCTGGGTTAACTGGGAAAGTGATGT

HindIII (1074)  
1001 GGTGACTGGCTCCACCTTTTTCCCAGGGTGGGGGAGAACCATATATAAGTGCAGTAGTCTGTGAACATTCAAGCTTCTGCCTTCTCCCTCCTGTGA  
1101 GTTTGtaagtcactgactgtctatgctgggaaaggggtgggcaggaggtggggcagtgaggaaaagtggcactgtgaacctgcagccctagacaatt

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 TCCACCAGAACCCAAATCAACAAGGTTGTAAGGTTTGACAAGCTGCCAGGCTTTGGTACTCAATAGAGGCCAGTGTGGCACCAGTGTAAATGTACACT  
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 TGGGTGGAGACCCAGACCAATGGAATAATCAGGAATGTTCTGAGCCTTCTATCTGACTCCAGACAGCAATGGTCTTGGTCAATGCAATTGTCTTCA  
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 AGGGCTGTGGGAGAAGCTTTCAAAGATGAAGACACTCAGGCAATGCCCTCAGAGTAACTGAACAGGAGTCCAAACCTGTGCAGATGATGTACCAAAT  
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 ACCTGCCAGAATGAAAATGGAGGAGAAAATACAACCTCACCTCAGTGTGATGGCAATGGGATAACAGATGTCTTCTCCAGCTCTGCCAACCTCTCTGG  
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 GGAGTGGATGCTGCCTCTGCTCAGAAGAGTTGAGAGCAGACCACCCCTTCTCTGATCAAGCATATAGCCACCAATGCTGTTCTTTCTTTGGAA  
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 GGAGGTGTGGGAGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTATGGAATTCAGTCAATATGTTACCCCAAAAAGCTGTTGTAACTTGC  
2701 AACCTCATTCTAAAATGTATATAGAAGCCAAAAGACAATAACAAAATATTCTTGTAGAACAAAATGGGAAAGAATGTTCCACTAAATATCAAGATTTA

SacI (2853)  
2801 GAGCAAAGCATGAGATGTGTGGGATAGACAGTGAAGGCTGATAAAATAGAGTAGAGCTCAGAAACAGACCCATTGATATATGTAAGTACCTATGAAAA

2901 AATATGGCATTTTACAATGGGAAATGATGGTCTTTTTCTTTTTAGAAAAACAGGGAATATATTTATATGTAAAAATAAAAGGGAACCCATATGTCA  
3001 TACCATACACACAAAAAATCCAGTGAATTATAAGTCTAAATGGAGAAGGCAAAACTTTAAATCTTTAGAAAATAATATAGAAGCATGCCATCAAGAC  
3101 TTCAGTGTAGAGAAAAATTTCTTATGACTCAAAGTCTAACCCACAAAGAAAAGATTGTTAATTAGATTGCATGAATATTAAGACTTATTTTTAAATTA  
3201 AAAACCATTAAGAAAAGTCAGGCCATAGAATGACAGAAAATATTTGCAACACCCAGTAAAGAGAATTGTAATATGCAGATTATAAAAAAGAGTCTTACA  
3301 AATCAGTAAAAATAAAACTAGACAAAAATTTGAACAGATGAAAGAGAACTCTAAATAATCATTACACATGAGAACTCAATCTCAGAAATCAGAGAAC

PacI (3458)

3401 TATCATTGCATATACACTAAATTAGAGAAATATTTAAAAGGCTAAGTAACATCTGTGGCTTAATTTAAATCAGCAGTTCAACCTGTTGATAGTATGTA  
3501 AGCTCTCATGTTAATGTACTAAGCTCTCATGTTAATGAACTAAACCCTCATGGCTAATGTACTAAGCTCTCATGGCTAATGTACTAAGCTCTCATGTT

AseI (3633)

3601 TCATGTAAGCTCTCATGTTTGAACAATAAAATTAATATAAATCAGCAACTTAAATAGCCTCTAAGGTTTTAAGTTTTATAAGAAAAAAGAATATA

3701 TAAGGCTTTAAAGGTTTAAGGTTTCCTAGCTTAGTCTGTTCTCAGCTACAAAATGGACACAATTTCCAGCAGGGTCTCTGAGGGCAAATTCCTT

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

3801 CCCAAGTTGTTACCAATTTCTGTCATGGCTGGGCCAGAGGCATCCCTGAAATTTGTGCTGACTACTTCTGACCATTCTGCATAAAGCTCATCTAGGC

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 CTCTGACCCAGACCAAGCAAGGGTGTGTCAGGGACAACCTGGCTGAACTGCTGAGATGAAGAGGGTGACATCATCTCTGACAACACCAGCAAAATC

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