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PRODUCT INFORMATION

Content:
- 20 µg of pCpGfree-mSEAP 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 for 1 year when stored at -20°C.
- Resuspended DNA is stable for 6 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. Viability of the lyophilized bacteria upon resuspension has been verified.

GENERAL PRODUCT USE

InvivoGen has developed a new 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.

pCpG 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.

pCpG plasmids represent valuable tools to study the effects of CpGs on gene expression in vivo and in vitro, using cell lines expressing TLR9, as well as their effects on the innate and acquired immune systems. Furthermore, for RNAi applications, InvivoGen has designed pCpG-siRNA a plasmid that allows long term production of siRNAs in vivo.

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.

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 λ, encoded by the pir gene).
- Bacterial promoter: EM2K is a CpG-free version of the bacterial EM7 promoter.
- Selectable marker: The Zeo3+t 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.
- Polyanadenylation signal: The polyanadenylation 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. pCpG plasmids contain 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-mSEAP expresses the synthetic mSEAP∆CpG gene, a CpG-free allele of the mSEAP gene constructed by chemical synthesis.

Due to the presence of the R6Kγ 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.


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 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-mSEAP 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 400 Watts), 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.
pCpGfree-mSEAP_v2
(4546 bp)

- pCpGfree
- IFNß S/MAR
- βGlo MAR
- mCMV enh
- hEF1 prom
- R6K ori
- Zeo
- EM2K
- SV40 pAn
- mSEAP

Restriction sites:
- PacI (3821)  
- Sacl (3216)  
- EcoRI (3015)  
- NheI (2781)  
- SacI (2548)  
- PshAI (2378)  
- SfiI (4465)  
- EcoRI (415)  
- SdaI (420)  
- SpeI (850)  
- HindIII (1074)  
- BsrGI (1238)  
- PshAI (2378)