pORF-mIL-12

An expression vector containing the murine IL-12 gene
Catalog # porf-mill12

For research use only
Version # 01E25-MT

PRODUCT INFORMATION

Content:
- 1 disk of lyophilized GT100 E. coli bacteria transformed by pORF-mIL12.
- GT100 genotype is: F-, mcrA, Δmrr-hsdRMS-mcrBC, Δ80lacZΔM15, ΔlacX74, recA1, endA1.
- 4 pouches of E. coli FastMedia™ Amp.

Storage and stability:
- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20°C and are stable up to 1 year.
- Store E. coli FastMedia™ Amp at room temperature. FastMedia™ is stable 18 months when stored properly.

Quality control:
- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.

GENERAL PRODUCT USE

pORF is a ready-made expression vector containing a gene of interest.

pORF may be used for:

Obtaining a gene to subclone into another vector. Two unique restriction sites flank the gene, allowing convenient excision. These restriction sites are compatible with many restriction sites contained in multiple cloning sites, thus facilitating subcloning.

Gene expression in mammalian cells. Cells may be transiently transfected with pORF. The secreted protein may be harvested in the cell culture supernatant as all secreted proteins in pORF possess a signal sequence.

mIL12 gene may be cut out by using Nco I and Nhe I enzymes
Nco I is compatible with BspHI and BspL11I.
Nhe I is compatible with XbaI, SpeI, and AvrII.

PLASMID FEATURES

• EF-1α/HTLV hybrid promoter is a composite promoter comprised of the Elongation Factor-1α (EF-1α) promoter and 5’ untranslated region of the Human T-Cell Leukemia Virus (HTLV). EF-1α utilizes a type 2 promoter that encodes for a “housekeeping” gene. The promoter is stronger than CMV and 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 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.

• Intronic₁₁₁₇ 5’UTR: InvivoGen utilizes an inducible promoter for the second transcriptional unit that is spliced out as an intron in mammalian cells. LacI expression causes overproduction of Lac repressor protein acting on the bacterial promoter to repress the expression of the gene. This safeguard is essential when the second transcription gene product is toxic to E. coli. Treatment with IPTG enables the expression of the second transcription unit in bacteria constitutively expressing LacI.

• mIL12 gene
Murine mIL12 gene (intronless ORF) from the ATG to the stop codon.
Size: 1620 bp

• SV40 pA: The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA. The efficiency of this signal was first described by Carswell et al.

• Ori colE1 is a minimal E. coli origin of replication with the same activity as the longer Ori.

• Amp (ampicillin resistance gene): The ampicillin resistance gene allows the selection of bacteria carrying the pORF plasmid.

REFERENCES

METHODS

Growth of pORF-transformed bacteria:
Use sterile conditions to do the following:
1- Resuspend the lyophilized E. coli by adding 1 ml of LB medium in the tube containing the disk. Let sit for 5 minutes. Mix gently by inverting the tube several times.
2- Streak bacteria taken from this suspension on an ampicillin LB agar plate prepared with the E. coli FastMedia™ Amp agar provided (see below).
3- Place the plate in an incubator at 37°C overnight.
4- Isolate a single colony and grow the bacteria in TB supplemented with ampicillin using the FastMedia™ Amp liquid provided (see below).
5- Extract the pORF plasmid DNA using the method of your choice.

Selection of bacteria with E. coli FastMedia™ Amp:
E. coli FastMedia™ Amp is a new, fast and convenient way to prepare liquid and solid media for bacterial culture by using only a microwave.
E. coli FastMedia™ Amp is a TB (liquid) or LB (solid) based medium with ampicillin, and contains stabilizers.
E. coli FastMedia™ Amp can be ordered separately (catalog code: # fas-am-l, fas-am-s, fas-am-x).

Method:
1- Pour the contents of a 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 FastMedia™.
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 FastMedia™ as the antibiotic will be permanently destroyed by the procedure.
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(4846 bp)