pDUO-hCD14/TLR4A
A plasmid coexpressing the human CD14 and TLR4A genes
Catalog # pduo-hcd14tlr4a
For research use only
Version # 15H11-MM

PRODUCT INFORMATION

Content:
- 20 µg pDUO-hCD14/TLR4 provided as lyophilized DNA
- 4 pouches of E. coli Fast-Media® Blas (2 TB and 2 Agar)

Shipping and storage:
- Products are shipped at room temperature.
- Lyophilized DNA should be stored at -20 °C
- Resuspended DNA should be stored at -20 °C and is stable up to 1 year. Avoid repeated freeze-thaw cycles.
- Store E. coli Fast-Media® Blas at room temperature. Fast-Media® pouches are stable 18 months when stored properly.

Quality control:
Plasmid construct has been confirmed by restriction analysis and sequencing.

GENERAL PRODUCT USE

Toll-Like receptors (TLRs) play a critical role in early innate immunity to invading pathogens by sensing microorganisms. These evolutionarily conserved receptors, homologues of the Drosophila Toll gene, recognize highly conserved structural motifs only expressed by microbial pathogens, called pathogen-associated microbial patterns (PAMPs). PAMPs include various bacterial cell wall components such as lipopolysaccharides (LPS), peptidoglycans and lipopeptides, as well as flagellin, bacterial DNA and viral double-stranded RNA. Stimulation of TLRs by PAMPs initiates a signaling cascade that involves a number of proteins, such as MyD88 and IRAK. This signaling cascade leads to the activation of the transcription factor NF-κB which induces the secretion of pro-inflammatory cytokines and effector cytokines that direct the adaptive immune response.

To date ten human and twelve murine TLRs have been characterized, TLR1 to TLR10 in humans, and TLR1 to TLR9, TLR11, TLR12 and TLR13 in mice, the homolog of TLR10 being a pseudogene. In many instances, TLRs require the presence of a co-receptor to initiate the signaling cascade. One example is TLR4 which interacts with MD2 and CD14 to induce NF-κB in response to LPS stimulation.

pDUO is an expression vector designed to co-express two TLRs or TLR-related genes known to interact with each other. The genes cloned into pDUO comprise the coding sequence (without introns) from the ATG to the Stop codon.

PLASMID FEATURES

- **Human CD14 (1125 bp)/Human TLR4A (2517 bp)**
  TLR4 is the receptor for Gram-negative lipopolysaccharide (LPS). The TLR4 gene was shown to be mutated in C3H/HeJ and C57BL/10ScCr mice, both of which are low responders to LPS. However, TLR4 alone is not sufficient to confer LPS responsiveness. TLR4 requires MD-2, a secreted molecule, to functionally interact with LPS. TLR4 physically associates with MD2, and together with a third protein called CD14, this complex is responsible for LPS recognition and signaling.

- **hFerH and hFerL composite promoters:** Ferritin is a 24 subunit protein composed of two subunit types, termed H (heavy) and L (light), which perform complementary functions in the protein. Ferritin is ubiquitously expressed. Its synthesis is highly regulated by the iron status of the cell. The iron regulation is achieved at the translational level through the interaction between the iron-responsive element (IRE), located in the 5’ untranslated region (5’UTR) of the ferritin mRNAs, and the iron regulatory protein. To eliminate the iron regulation of the ferritin promoters, the 5’UTR of FerH and FerL have been replaced by the 5’UTR of the mouse and chimpanzee elongation factor 1 (EF1) genes, respectively.

- **SV40 enhancer** which is comprised of a 72-base-pair repeat allows the enhancement of gene expression in a large host range. The enhancement varies from 2-fold in non-permissive cells to 20-fold in permissive cells. Furthermore, the SV40 enhancer is able to direct nuclear localization of plasmids.

- **SV40 pAn:** 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.

- **CMV enhancer:** The major immediate early enhancer of the human cytomegalovirus (HCMV), located between nucleotides -118 and -524, is composed of unique and repeated sequence motifs. The HCMV enhancer can substitute for the 72-bp repeats of SV40 and is severalfold more active than the SV40 enhancer.

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- **pMB1 ori:** a minimal E. coli origin of replication to limit vector size, but with the same activity as the longer Ori.

- **FMDV IRES:** The internal ribosome entry site of the Foot and Mouth Disease Virus enables the translation of two open reading frames from one mRNA with high levels of expression.

TECHNICAL SUPPORT
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EM7 is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in E. coli.

Bsr (blasticidin resistance gene): The bsr gene from Bacillus cereus encodes a deaminase that confers resistance to the antibiotic Blasticidin S. In bacteria, bsr is expressed from the constitutive E. coli EM7 promoter. In mammalian cells, bsr is transcribed from the human FerH composite promoter as a polycistronic mRNA and translated via the FMDV IRES.

EF1 pAn is a strong polyadenylation signal. InvivoGen uses a sequence starting after the stop codon of the EF1 cDNA and finishing after a bent structure rich in GT.

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.

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.

Selection of bacteria with E. coli Fast-Media Blas:

E. coli Fast-Media® Blas is a fast and convenient way to prepare liquid and solid media for bacterial culture by using only a microwave.

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 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 take 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.

**References:**
