pDRIVE5SEAP-hCD68
A plasmid with a native tissue-specific human CD68 promoter
Catalog # pdrive5s-hcd68
For research use only
Version # 14F11-MM

PRODUCT INFORMATION
Content:
- 20 µg of pDRIVE5s-hCD68 provided as lyophilized DNA
- 4 pouches of E. coli Fast-Media® Zeo (2 TB and 2 Agar)
Shipping and storage:
- Products are shipped at room temperature.
- Lyophilized DNA is stable for at least 12 months when stored at -20ºC
- Resuspended DNA is stable for 6 months when stored at -20ºC. Avoid repeated freeze-thaw cycles.
- Store E. coli Fast-Media® Zeo 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
pDRIVE5-SEAP is an expression plasmid containing a native or composite promoter of interest. pDRIVE5-SEAP may be used to:
- Subclone a promoter of interest into another vector. Unique restriction sites are present at each end of the promoter allowing convenient excision. The 5’ sites are Sdi I and Spe I. Sdi I is compatible with Nsi I and Pst I. Spe I is compatible with Avr II, Nhe I and Hha I. The 3’ restriction site is Nco I which includes the ATG start codon, and is compatible with BspF I and BspLU11 I.
- Compare the activity of different promoters in transient transfection experiments. Each pDRIVE5-SEAP promoter drives the expression of the SEAP reporter gene which allows for testing of the promoter’s activity in transient transfection experiments. Furthermore, the SEAP gene is flanked by unique restriction sites (Nco I and Nhe I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS
Human CD68 promoter
Complete Promoter Size: 663 bp
Specificity: Macrophages
Murine macrosialin and its human homolog CD68 are heavily glycosylated transmembrane proteins expressed specifically in macrophages and macrophage-related cells. The CD68 gene promoter directs macrophage-specific expression. Although the promoter lacks a classical TATA box, it contains other protein binding sites consistent with preferential monocyte/macrophage gene expression1. CD68 promoter has been used to achieve constitutive expression of IL-10 specifically in macrophages1.


PLASMID FEATURES
- SEAP gene encodes an engineered secreted embryonic alkaline phosphatase. The levels of SEAP in the culture medium of transfected cells expressing the reporter gene can be assayed with chromogenic or luminescent methods
- SV40 pAn: The Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
- pMB1 Ori is a minimal E. coli origin of replication with the same activity as the longer Ori.
- EM2K is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in E. coli.
- Zeo gene confers zeocin resistance therefore allowing the selection of transformed E. coli carrying a pDRIVE5-SEAP plasmid.

Note: Stable transfection of clones cannot be performed due to the absence of an eukaryotic promoter upstream of the Sh ble gene.

METHODS
Plasmid resuspension:
Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1µg/µl, resuspend the DNA in 20µl of sterile H2O. Store resuspended plasmid at -20ºC.

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 Zeo:
E. coli Fast-Media® Zeo is a fast and convenient way to prepare liquid and solid media for bacterial culture by using only a microwave. E. coli Fast-Media® Zeo is a TB (liquid) or LB (solid) based medium with zeocin, and contains stabilizers. E. coli Fast-Media® Zeo can be ordered separately (catalog code # fas-zn-l, fas-zn-s).

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 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 45ºC before pouring plates. Let liquid media cool to 37ºC 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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pDRIVE5s-hCD68
(4177 bp)
| 3501 | TCCGCCCTCTGACAGCATCACAATAACGACGTCAAGTCAGAGTGCGGAAACCGGACAGGACTATAAGGATACGACGCTTTCCCCCTGGAAGCTC |
| 3601 | CCTCGTCGCTCTCTGTTGGACCCCTGCGCTTTACCGATACCTGTCGCCCTTTTCTCCTCGAAGCCGTGGCTTTCTCATAGCTACGCCTGTAAGG |
| 3701 | TATCTCAGTTCTCGGTAGGTCGCTCGCCGCTCAAGCTGGGCTGTCGCGAAGCCCGCGCTGCCTTTAATCCGGAACGTATCGCTCTTG |
| 3801 | AGTCCAACCCGCTAAGACAGACTTATGCGCAGACGCCACTGCTAACAGATTACAGACGGTATGTTGGCCGGTGCTACAGAGTTCTTGAA |
| 3901 | GTGGTGCCCTAACTACGCTACACTAGAAAGCAGATTGGTACTCTCGCCTCTGCTGAAAGCCAGTTTACCTCCTGGAAAAGAGTTGGTGACTGCTTGATCC |
| 4001 | GGCAACCAACCCCGCTGGTAGCCGCGCTGTTTTTTGTCTTGCAAGCAGCAGATTACGCCAGAAGGATCCTCAAGAAGATTCTTTTGATTCTTTCTA |
| 4101 | CGGGGTGTAGCGCTAGGACGAAACTCAGCGTAAAGGGATTGTGTCTGATGTTAATTAACTATTAAATCA |