**pDRIVE-hCD68**

A plasmid with the native human CD68 promoter

Catalog # pdrive-hcd68

For research use only

Version # 14F11-MMv02

**PRODUCT INFORMATION**

**Content:**
- 20 µg of pDRIVE-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 -20°C.
- Resuspended DNA is stable for 6 months when stored at -20°C. 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**

pDRIVE is an expression plasmid containing a native or composite promoter of interest. pDRIVE 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 include Sda I, Pst I, and Spe I. Sda I is compatible with Nsi I and Pst I. Spe I is compatible with Avr II, Nhe I and Xho I. The 3’ restriction site is Nco I which includes the ATG start codon, and is compatible with BspHI I and BspLU11 I.
- **Compare the activity of different promoters in transient transfection experiments.** Each pDRIVE promoter drives the expression of the LacZ reporter gene which allows for testing of the promoter’s activity in transient transfection experiments. Furthermore, the LacZ gene is flanked by unique restriction sites (Nco I and EcoR 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 expression. The CD68 promoter has been used to achieve constitutive expression of IL-10 specifically in macrophages.


**PLASMID FEATURES**

- **LacZ gene** encodes β-galactosidase an enzyme that catalyzes the hydrolysis of X-Gal, producing a blue precipitate that can be easily visualized under a microscope.
- **SV40 pA N:** 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.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Sh ble** gene confers zeocin resistance therefore allowing the selection of transformed *E. coli* carrying a pDRIVE plasmid.

**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 -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 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 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.