PRODUCT INFORMATION

Contents:
- 20 µg of pDRIVE5SEAP-hCXCR4 provided as lyophilized DNA
- 4 pouches of E. coli Fast-Media® Zeo (2 TB and 2 Agar)

Storage and Stability:
- Product is shipped at room temperature.
- Lyophilized DNA should be stored at -20 ºC.
- Resuspended DNA should be stored at -20 ºC and is stable up to 1 year.
- Store E. coli Fast-Media® at room temperature in a dry and cool place. Fast-Media® pouches are stable 2 years when stored properly.

Quality Control:
- Plasmid construct has been confirmed by restriction analysis and full-length ORF sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

GENERAL PRODUCT USE

pDRIVE5SEAP is an expression plasmid containing a native or composite promoter of interest. pDRIVE5SEAP 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’ site are SdiI and SpeI. SdiI is compatible with NsiI and PstI. SpeI is compatible with Avr II, Nhe I and Xba I. The 3’ restriction site is NcoI which includes the ATG start codon, and is compatible with BspHI and BspLU111.
- Compare the activity of different promoters in transient transfection experiments. Each pDRIVE5SEAP 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 restriction sites (NcoI and Nhel) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Human CXC-Chemokine receptor 4 promoter
Complete promoter size: 279 bp

Specificity: Tumor specific

CXCR4 is the receptor for the chemokine SDF1 and a coreceptor for HIV-1 entry. The expression of CXCR4 is upregulated in many types of cancers but low in the liver and other major organs. The CXCR4 promoter contains a TATAbox, a nuclear respiratory factor-1 (NRF-1) site, and for GC boxes1. A 279 bp fragment of the CXCR4 promoter was shown to have a “tumor-on” status in vitro and in vivo particularly in melanoma and breast cancers2.

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 pDRIVE 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 1 µg/µl, resuspend the DNA in 20 µl of sterile H2O. Store resuspended plasmid at -20 ºC.

Plasmid amplification and cloning:
Plasmid amplification and cloning can be performed in E. coli GT116 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 ºC before pouring plates. Let liquid media cool to 37 ºC before seeding bacteria.

Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.