**pDRIVE-mGFAP**

A plasmid encoding the native tissue specific murine GFAP promoter

Catalog # pdrive-mgfap

For research use only

Version # 15116-MM

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**PRODUCT INFORMATION**

**Contents:**
- 20 µg of pDRIVE-mGFAP 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 °C.
- Resuspended DNA should be stored at -20 °C 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**

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’ site is SdaI and SpeI. SdaI is compatible with NsiI and PstI. SpeI is compatible with AvrII, NheI and XbaI. SdaI is compatible with NsiI and PstI. The 3′ restriction site is NcoI which includes the ATG start codon, and which is compatible with BspHI and BspLU111.
- 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.
- 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 is SdaI and SpeI. SdaI is compatible with NsiI and PstI. SpeI is compatible with AvrII, NheI and XbaI. SdaI is compatible with NsiI and PstI. The 3′ restriction site is NcoI which includes the ATG start codon, and which is compatible with BspHI and BspLU111.

**PROMOTER CHARACTERISTICS**

**Murine Glial Fibrillary Acidic Protein promoter**

Complete Promoter size: 1679 bp

Specificity: Astrocytes

The glial fibrillary acidic protein (GFAP) is an intermediate filament protein found almost exclusively in astrocytes. It is expressed throughout postnatal life and is upregulated in response to almost any damage to the central nervous system, including Parkinson’s disease. The promoter of the GFAP gene was shown to direct astrocyte-specific transcription in *vivo* and in *vitro*, and in transgenic mice. Expression of a transgene under the control of the GFAP promoter is regulated in a similar fashion as the endogenous GFAP gene.


**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 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 °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.