**pDRIVE-rGFAP**

A plasmid encoding the native tissue specific rat GFAP promoter

Catalog # pdrive-rgfap

**For research use only**

Version # 15116-MM

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**PRODUCT INFORMATION**

Contents:
- 20 µg of pDRIVE-rGFAP 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 and is stable up to 1 year.
- *E. coli* Fast-Media® pouches are stable 2 years when stored properly.
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Quality Control:
- Plasmid construct has been confirmed by restriction analysis and full-length ORF sequencing.
- Plasmid DNA was purified by ion exchange chromatography.

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**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. SdaI is compatible with NsiI and PstI. The 3′ sites are present at each end of the promoter allowing convenient 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 (BspHI and EcoRI) for easy replacement with a different gene of interest.

**PROMOTER CHARACTERISTICS**

Rat Glial Fibrillary Acidic Protein promoter

Complete Promoter size: 1589 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 *vitro*, *in vivo*, 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.

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**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.

**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 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-tb, 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.

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**TECHNICAL SUPPORT**

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