pDRIVE5SEAP-mGFAP
A plasmid encoding the native tissue specific murine GFAP promoter
Catalog # pdrive5s-mgfap

For research use only
Version # 15J16-MM

PRODUCT INFORMATION
Contents:
• 20 µg of pDRIVE5SEAP-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 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. Typically the 5’ sites are SdaI and SpeI. SdaI 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 BspLU11I.
- 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
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 vitro, in vivo1, and in transgenic mice1. Expression of a transgene under the control of the GFAP promoter is regulated in a similar fashion as the endogenous GFAP gene1.


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.
pDRIVE5s-mGFAP
(5196 bp)

EM2K
Zeo
SV40 p(A)
SEAP
pr. (m)GFAP
ori pMB1

XbaI (19)
EcoRI (23)
NotI (2)
SpeI (45)
SdaI (38)
Acc65I (615)
SphI (660)
NcoI (1726)
SacII (2708)
SphI (1748)

AatII (4275)
SgrAI (4199)
MfeI (3441)
NheI (3292)
MfeI (4362)
SspI (3771)
SspI (3771)
SspI (3771)
SspI (3771)
Fast-Media®

Microwaveable media for selection and propagation of *E. coli* transformants
Catalog # fas-xx-l, fas-xx-s, fas-xx-xgal

For research use only
Version # 10G07-MM

**PRODUCT INFORMATION**

**Contents:**
*E. coli* Fast-Media® are prepared as individual sealed pouches containing the necessary amount of powder for preparation of 200 ml of selective liquid or agar medium.

30 pouches are supplied for each order of TB or Agar and 20 pouches are supplied for each order of XGal Agar.

**Storage and stability:**
Fast-Media® are shipped at room temperature, and must be stored in a dry and cool place. They are stable for 2 years at room temperature.

When properly prepared, Fast-Media® plates or broths are stable for 4 weeks at 4°C, and remain sterile and selective.

**Quality control:**
The high quality and performance of each formulation has been tested with some widely used and proprietary *E. coli* K12 derived strains*. These include DH5α, Top10, MC1061, XL1 blue, JM 109, TB1, GT100, GT110, GT115, GT116.

The adequate plasmids carrying the appropriate *E. coli* resistance genes are used as positive control.

*E. coli* recipient strains carrying the Tn5 transposon are resistant to Kanamycin and Zeocin™.

**GENERAL PRODUCT USE**

*E. coli* Fast-Media® are microwaveable ready-to-use solid or liquid media, supplied with a selective antibiotic, and chromogenic substrates (for five references), therefore designed for the growth or selection of *E. coli* transformant colonies, as well as detection of blue/white colonies.

- **Fast-Media® Agar** formulation is LB based agar medium supplemented with selective antibiotic, it is used for selection of resistant *E. coli* colonies after transformation by vectors carrying a selection resistance gene.

- **Fast-Media® X-Gal** formulation is a LB based agar medium supplemented with selective antibiotic, X-Gal and IPTG. It is used for detection of blue/white resistant colonies after transformation by a vector carrying LacZ gene.

- **Fast-Media® TB** formulation is a Terrific Broth based liquid medium supplemented with selective antibiotic. It’s used for high cell density culture of transformed bacteria, and extraction of high quantity and quality of required plasmid.

**FAST-MEDIA® FEATURES**

*E. coli* Fast-Media® offer researchers a quick and convenient way to prepare 200 ml of liquid culture medium, or 8-10 agar plates in about five minutes USING A MICROWAVE INSTEAD OF AN AUTOCLAVE.

*E. coli* Fast-Media® are available with a large variety of prokaryotic selective agents including Ampicillin, Blasticidin S, Hygromycin B, Kanamycin, Puromycin and Zeocin™ (see table below). Fast-Media® is also available with no selective agent (Base) that can be prepared with or without antibiotics.

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**SPECIAL HANDLING**

Caution should be exercised during handling of Fast-Media® due to potential allergenic properties of antibiotics. Wear protective gloves, do not breath the dust.

**METHOD**

For customer convenience, procedure is directly printed on each pouch.

1- Pour the pouch contents into a clean borosilicate glass bottle or flask.
2- Add 200 ml of distilled or deionized water.
3- Mix thoroughly by swirling the glass bottle or flask.
4- Heat in a microwave oven on MEDIUM power setting (about 450W) until bubbles start to appear (about 3 minutes).

**Do not heat in a closed container.**

5- Swirl gently to mix the preparation and re-heat for 30 seconds. Swirl gently again.
6- Repeat step 4 if necessary until the medium is completely dissolved. Do not overboil.
7- Allow the medium to cool to 50-55 °C, use directly for liquid medium, or pour plates for solid medium.

**Caution:** Any solution heated in a microwave oven may become superheated and suddenly boil when moved or touched. Handle with extreme care. Wear heat-proof gloves.

**Note:** Do not repeat this above procedure once the medium is prepared because the antibiotic will be adversely affected.

For preparation of supplemented Fast-Media® Base.

- Follow the instructions above and when media has cooled to 50-55 °C add the antibiotic at the appropriate concentration for selection of *E. coli*.

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**TECHNICAL SUPPORT**
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