pDRIVE-hGAPDH

A plasmid with the native human GlycerAldehyde 3-Phosphate DeHydrogenase promoter

Catalog # pdrive-hgapdh

For research use only

Version # 01E02-MT

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT100 *E. coli* bacteria transformed by a pDRIVE plasmid.
- GT100 genotype is: *F-, mcrA, Δ(mrr-hsdRMS-mcrBC), Ø80lacZΔM15, ΔlacX74, recA1, endA1*.
- 4 pouches of *E. coli* FastMedia™ Zeo

Shipping and storage:

- Products are shipped at room temperature.
- Transformed bacteria should be stored at -20˚C. Bacteria are stable up to one year when properly stored.
- Store *E. coli* FastMedia™ Zeo at room temperature. FastMedia™ is stable 18 months when stored properly.

Quality control:

- Plasmid construct has been confirmed by restriction analysis and sequencing.
- Bacteria have been lyophilized, and their viability upon resuspension has been verified.
- Promoter activity has been confirmed by transient transfection of 293 cells as well as other selected cell lines.

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 *Xba* I. The 3'restriction site is *Nco* I which includes the ATG start codon, and is compatible with *BspH* I and *BspL*U11 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

Element	Name	Origin	Size bp
Promoter	GAPDH	Human	482
5'UTR	GAPDH	Human	313
Enhancer	-	-	-

GAPDH promoter

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a catalytic enzyme involved in glycolysis. The GAPDH gene is constitutively expressed at high levels in almost all tissues. However, the molecular mechanism which sustains high-level expression of this house-keeping enzyme is still unclear. The expression of GAPDH can be induced by several physiological factors in certain cell types. In adipocytes, insulin increases the levels of GAPDH mRNA through cis-acting sequences located within the GAPDH promoter[1]. In endothelial cells, GAPDH is induced by hypoxia at the transcriptional level, this upregulation occuring to a much greater extent in EC than in other cell types[2].

PLASMID FEATURES

• **LacZ gene** encodes ß-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.

• **Ori pMB1** 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.

Note: *Stable transfection of clones cannot be performed due to the absence of an eukaryotic promoter upstream of the Sh ble gene.*

METHODS

Growth of pDRIVE-transformed bacteria:

Use sterile conditions to do the following:

1- Resuspend the lyophilized *E. coli* by adding 1 ml of LB medium in the tube containing the disk. Let sit for 5 minutes. Mix gently by inverting the tube several times.

2- Streak bacteria taken from this suspension on a zeocin LB agar plate prepared with the *E. coli* FastMedia™Zeo agar provided (see below).

3- Place the plate in an incubator at 37˚C overnight.

4- Isolate a single colony and grow the bacteria in TB supplemented with zeocin using the FastMedia™ Zeo liquid provided (see below).

5- Extract the pDRIVE plasmid DNAusing the method of your choice.

Selection of bacteria with *E. coli* FastMedia™ Zeo:

E. coli FastMedia™ Zeo is a **new, fast and convenient** way to prepare liquid and solid media for bacterial culture by using only a microwave. *E. coli* FastMedia™ Zeo is a TB (liquid) or LB (solid) based medium with zeocin, and contains stabilizers.

E. coli FastMedia™ 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 FastMedia™.**

4- Swirl gently to mix the preparation. **Be careful, the bottle and media are hot, use heatproof pads orgloves 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 FastMedia™ as the antibiotic will be permanently destroyed by the procedure.*

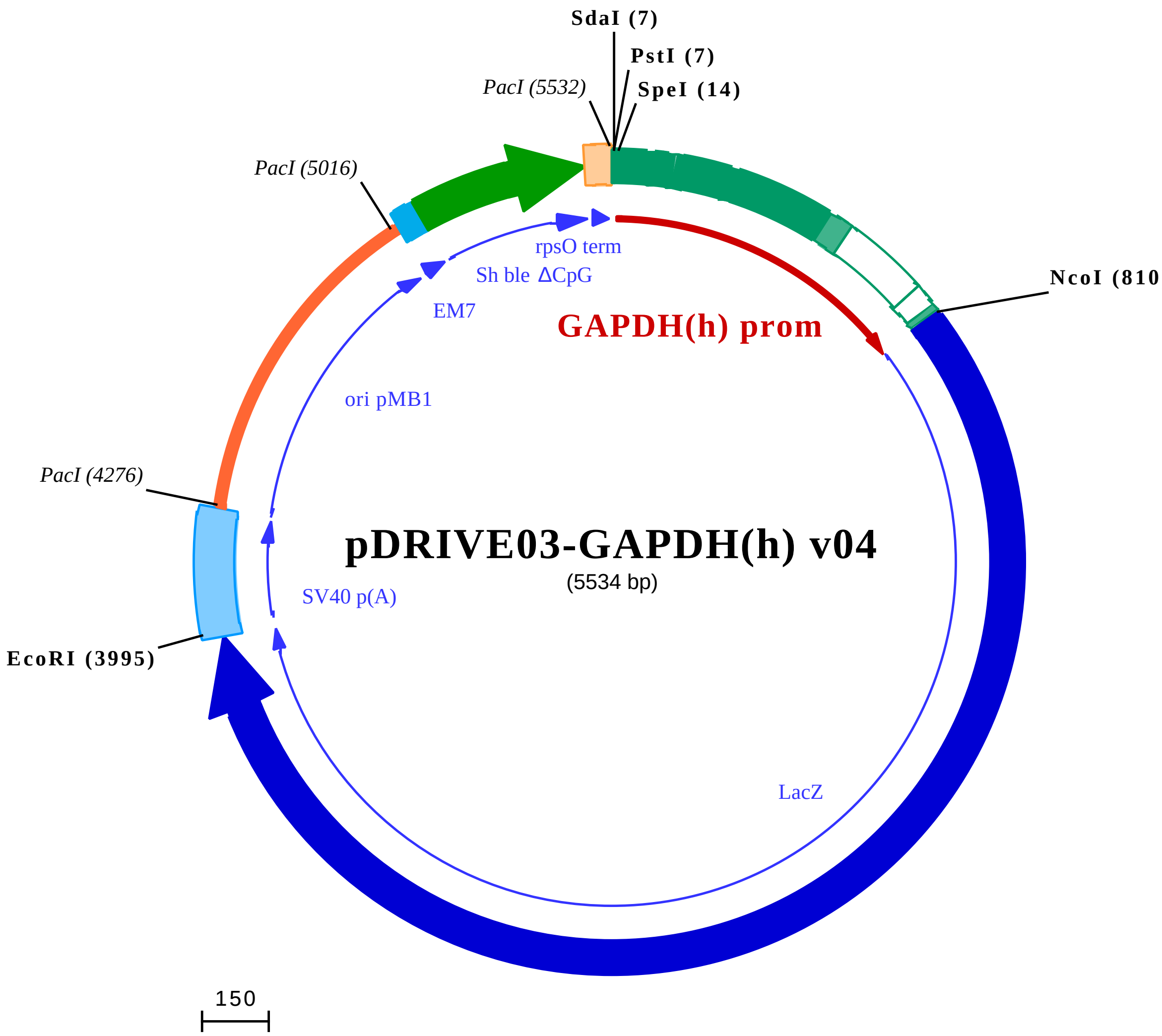
References:

1- Ercolani Let *al.* 1988. J Biol Chem. 263(30):15335-41

2- Graven KK et al. 1999. Biochim Biophys Acta. 1447(2-3):208-18

TECHNICAL SUPPORT
Toll free (US): 888-457-5873
Outside US: (+1) 858-457-5873
E-mail: info@invivogen.com
Website: www.invivogen.com

InvivoGen
3950 Sorrento Valley Blvd. Suite A
San Diego, CA92121 - USA


SdaI (7)
PstI (7)
SpeI (14)
PacI (5532)
PacI (5016)
NcoI (810)
rpsO term
Sh ble ΔCpG
EM7
GAPDH(h) prom
ori pMB1
PacI (4276)
pDRIVE03-GAPDH(h) v04
(5534 bp)
SV40 p(A)
EcoRI (3995)
LacZ
150

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      PstI (7)
      SdaI (7)       SpeI (14)
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 201 CAGTCCAGTCCTGGGAACCAGCACCGATCACCTCCCATCGGGCCAATCTCAGTCCCTTCCCCCCTACGTCGGGGCCCACACGCTCGGTGCGTGCCCAGTT
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 601 ggggcgggcgcaggccggatgtgttcgcgccgctgcggggtgggcccgggcggcctccgcattgcaggggcgggcggaggacgtgatgcggcgcgggctg
 701 ggcatggaggcctggtgggggaggggaggggaggcgtgggtgtcggccggggccactaggcgctcactgttctctccctccgcgcagCCGAGCCACATCG
                NcoI (810)
 801 CTGAGACACCATGGGGGGTTCTCATCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGGGATCTGTACGACGATGACGAT
   1 MetGlyGlySerHisHisHisHisHisHisGlyMetAlaSerMetThrGlyGlyGlnGlnMetGlyArgAspLeuTyrAspAspAspAsp
 901 AAGGTACCTAAGGATCAGCTTGGAGTTGATCCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATC
  31 LysValProLysAspGlnLeuGlyValAspProValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGlnLeuAsnArgLeuAlaAlaHisP
1001 CCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCC
  64 roProPheAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPhePr
1101 GGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCG
  97 oAlaProGluAlaValProGluSerTrpLeuGluCysAspLeuProGluAlaAspThrValValValProSerAsnTrpGlnMetHisGlyTyrAspAla
1201 CCCATCTACACCAACGTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATG
 131 ProIleTyrThrAsnValThrTyrProIleThrValAsnProProPheValProThrGluAsnProThrGlyCysTyrSerLeuThrPheAsnValAspG
1301 AAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTAACTCGGCGTTTCATCTGTGGTGCAACGGGCGCTGGGTCGGTTACGGCCAGGA
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 197 pSerArgLeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValMetValLeuArgTrpSerAspGlySerTyrLeu
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1801 CCGAAACTGTGGAGCGCCGAAATCCCGAATCTCTATCGTGCGGTGGTTGAACTGCACACCGCCGACGGCACGCTGATTGAAGCAGAAGCCTGCGATGTCG
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