pDRIVE-hβ-Actin

A plasmid with a native human β-Actin promoter

Catalog # pdrive-hbact

For research use only

Version # 05A17-SV

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* Fast-Media® 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* Fast-Media® Zeo at room temperature. Fast-Media® pouches are 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 are *Sda* 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
Core promoter	β-actin	Human	1454
5'UTR	β-actin	Human	933
Enhancer	-	-	-

β-Actin promoter

Beta-actin is a highly conserved protein ubiquitously expressed in all eukaryotic cells as a component of the cytoskeleton and as a mediator of internal cell motility. A 1.2-kb fragment of the β-actin 5' flanking region is sufficient for efficient transcription[1]. This 1.2-kb fragment contains a TATA box, a CCAAT box and two highly conserved elements. This fragment was shown to drive high levels of expression of a transgene *in vitro*[2]. InvivoGen provides a larger fragment that includes the 5'UTR of the β-actin gene.

References:

1. Sugiyama H. *et al*. 1988. Strong transcriptional promoter in the 5' upstream region of the human beta-actin gene. Gene 65(1):135-9
2. Muller SR. *et al*. 1990. Efficient transfection and expression of heterologous genes in PC12 cells. DNA Cell Biol 9(3):221-9

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.

• **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* Fast-Media® 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 Fast-Media® Zeo liquid provided (see below).

5- Extract the pDRIVE plasmid DNA using the method of your choice.

Selection of bacteria with *E. coli* Fast-Media Zeo:

E. coli Fast-Media® Zeo is a **new, 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*.

TECHNICAL SUPPORT
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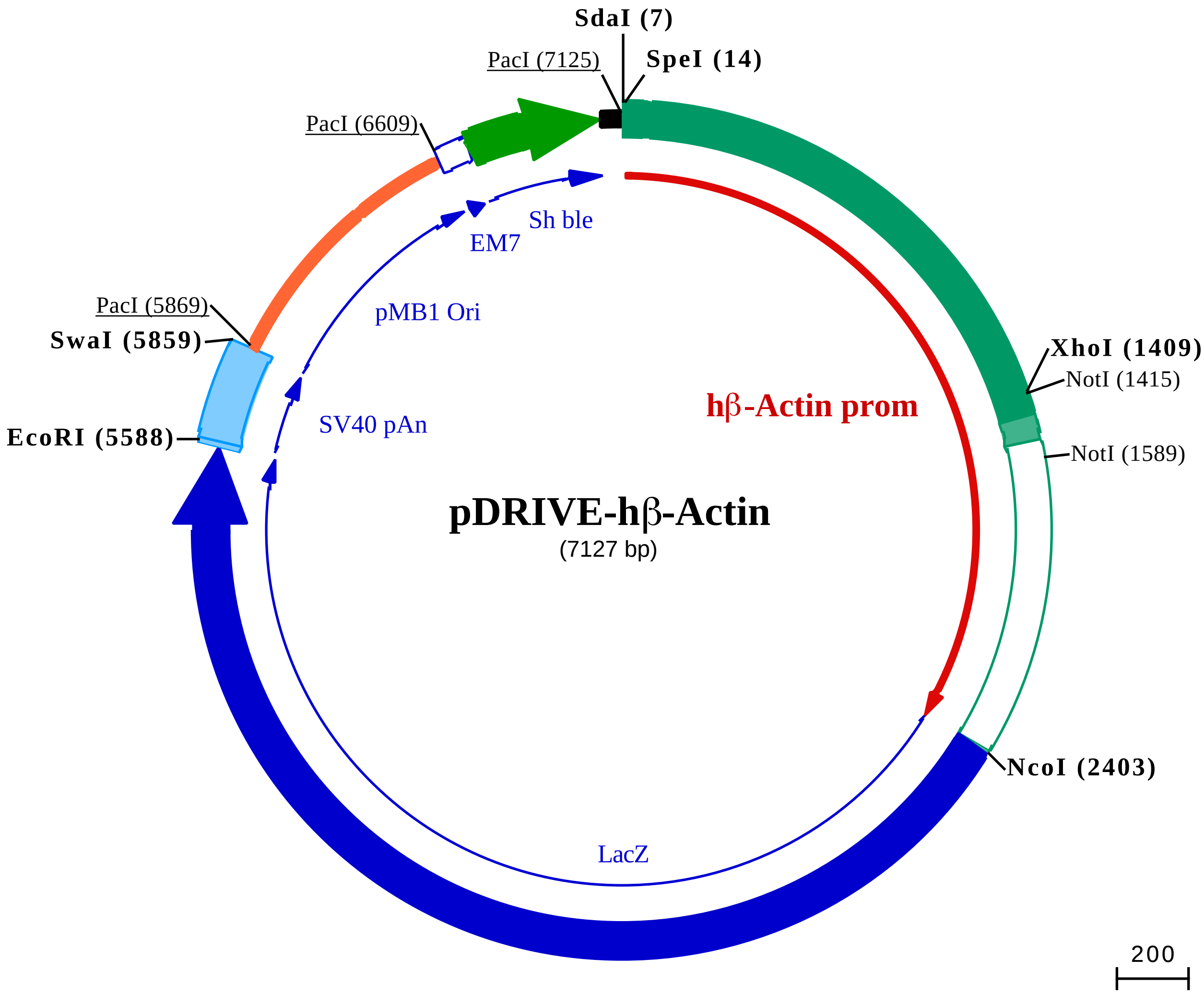
SdaI (7)
SpeI (14)
PacI (7125)
PacI (6609)
Sh ble
EM7
pMB1 Ori
PacI (5869)
SwaI (5859)
SV40 pAn
EcoRI (5588)
XhoI (1409)
NotI (1415)
NotI (1589)
hβ-Actin prom
pDRIVE-hβ-Actin
(7127 bp)
NcoI (2403)
LacZ
200

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     SdaI (7)        SpeI (14)
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 101 AGGCCTGAGGTGCCCCCACCTCACCACTCTTCCTATTTTTGTGTAAAAATCCAGCTTCTTGTCACCACCTCCAAGGAGGGGGAGGAGGAGGAAGGCAGGT
 201 TCCTCTAGGCTGAGCCGAATGCCCCTCTGTGGTCCCACGCCACTGATCGCTGCATGCCCACCACCTGGGTACACACAGTCTGTGATTCCCGGAGCAGAAC
 301 GGACCCTGCCCACCCGGTCTTGTGTGCTACTCAGTGGACAGACCCAAGGCAAGAAAGGGTGACAAGGACAGGGTCTTCCCAGGCTGGCTTTGAGTTCCTA
 401 GCACCGCCCCGCCCCCAATCCTCTGTGGCACATGGAGTCTTGGTCCCCAGAGTCCCCCAGCGGCCTCCAGATGGTCTGGGAGGGCAGTTCAGCTGTGGCT
 501 GCGCATAGCAGACATACAACGGACGGTGGGCCCAGACCCAGGCTGTGTAGACCCAGCCCCCCCGCCCCGCAGTGCCTAGGTCACCCACTAACGCCCCAGG
 601 CCTGGTCTTGGCTGGGCGTGACTGTTACCCTCAAAAGCAGGCAGCTCCAGGGTAAAAGGTGCCCTGCCCTGTAGAGCCCACCTTCCTTCCCAGGGCTGCG
 701 GCTGGGTAGGTTTGTAGCCTTCATCACGGGCCACCTCCAGCCACTGGACCGCTGGCCCCTGCCCTGTCCTGGGGAGTGTGGTCCTGCGACTTCTAAGTGG
 801 CCGCAAGCCACCTGACTCCCCCAACACCACACTCTACCTCTCAAGCCCAGGTCTCTCCCTAGTGACCCACCCAGCACATTTAGCTAGCTGAGCCCCACAG
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1101 GGTGGGTGGGGGGCCCGAGAGACGGGCAGGCCGGGGGCAGGCCTGGCCATGCGGGGCCGAACCGGGCACTGCCCAGCGTGGGGCGCGGGGGCCACGGCGC
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                         NotI (1415)
               XhoI (1409)
1401 TTTATGGCTCGAGCGGCCGCGGCGGCGCCCTATAAAACCCAGCGGCGCGACGCGCCACCACCGCCGAGACCGCGTCCGCCCCGCGAGCACAGAGCCTCGC
                                                                                                NotI (1589)
1501 CTTTGCCGATCCGCCGCCCGTCCACACCCGCCGCCAGgtaagcccggccagccgaccggggcaggcggctcacggcccggccgcaggcggccgcggcccc
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      NcoI (2403)
2401 ACCATGGGGGGTTCTCATCATCATCATCATCATGGTATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGGGATCTGTACGACGATGACGATAAGGTAC
   1 MetGlyGlySerHisHisHisHisHisHisGlyMetAlaSerMetThrGlyGlyGlnGlnMetGlyArgAspLeuTyrAspAspAspAspLysValP
2501 CTAAGGATCAGCTTGGAGTTGATCCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTT
  33 roLysAspGlnLeuGlyValAspProValValLeuGlnArgArgAspTrpGluAsnProGlyValThrGlnLeuAsnArgLeuAlaAlaHisProProPh
2601 CGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCA
  66 eAlaSerTrpArgAsnSerGluGluAlaArgThrAspArgProSerGlnGlnLeuArgSerLeuAsnGlyGluTrpArgPheAlaTrpPheProAlaPro
2701 GAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCT
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 200 LeuProSerGluPheAspLeuSerAlaPheLeuArgAlaGlyGluAsnArgLeuAlaValMetValLeuArgTrpSerAspGlySerTyrLeuGluAspG
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3201 TGATTTCAGCCGCGCTGTACTGGAGGCTGAAGTTCAGATGTGCGGCGAGTTGCGTGACTACCTACGGGTAACAGTTTCTTTATGGCAGGGTGAAACGCAG
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                                                                                                     EcoRI (5588)
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                                                                                   PacI (5869)
                                                                          SwaI (5859)
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