

pDRIVE5s-hEGR1

A plasmid with a ubiquitous human Early Growth Response 1 promoter

Catalog # pDRIVE5s-hegr1

For research use only

Version # 12D30-MM

PRODUCT INFORMATION

Content:

- 1 disk of lyophilized GT116 *E. coli* bacteria transformed by a pDRIVE5s plasmid.
- GT116 genotype is: *F*⁻, *mcrA*, Δ (*mrr-hsdRMS-mcrBC*), Δ 80*lacZ* Δ M15, Δ *lacX74*, *rspL* (*StrA*), *recA1*, *endA1* Δ *dem* Δ *sbcC-sbcD*.
- 4 pouches of *E. coli* Fast-Media® Zeo (2 TB and 2 Agar)

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.

GENERAL PRODUCT USE

pDRIVE5s is an expression plasmid containing a native or composite promoter of interest. pDRIVE5s 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 *BspLU11* I.
- **Compare the activity of different promoters** in transient transfection experiments. Each pDRIVE5s 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 unique restriction sites (*Nco* I and *Nhe* I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Human EGR1 promoter

Complete Promoter size: 1061 bp

Specificity: Ubiquitous, radiation inducible

Egr-1 is a member of the early growth response gene family like the c-fos and c-jun protooncogenes. Egr-1 expression is induced in many types of cells by various mitogenic stimuli or malignant transformation signals. The Egr-1 promoter is known to be transiently activated by ionizing radiation.

The radiation responsiveness is conferred by four CArG elements grouped in the 5' distal region of the promoter¹. The radiation inducibility of the Egr-1 promoter has been exploited in several experimental gene therapy strategies. In one of them, ionizing radiation was able to increase the expression of HSV1-tk 15- to 28-fold in human hepatocellular carcinoma *in vitro* and to significantly reduce the tumor size in nude mice².

1. Datta R. *et al.* 1992. Ionizing radiation activates transcription of the EGR1 gene via CArG elements. *Proc Natl Acad Sci USA*. 89:10149-53.
2. Kawashita Y. *et al.* 1999. Regression of hepatocellular carcinoma *in vitro* and *in vivo* by radiosensitizing suicide gene therapy under the inducible and spatial control of radiation. *Hum Gene Ther*. 10(9):1509-19.

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 pDRIVE5s 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 pDRIVE5s-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 pDRIVE5s 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

Toll free (US): 888-457-5873

Outside US: (+1) 858-457-5873

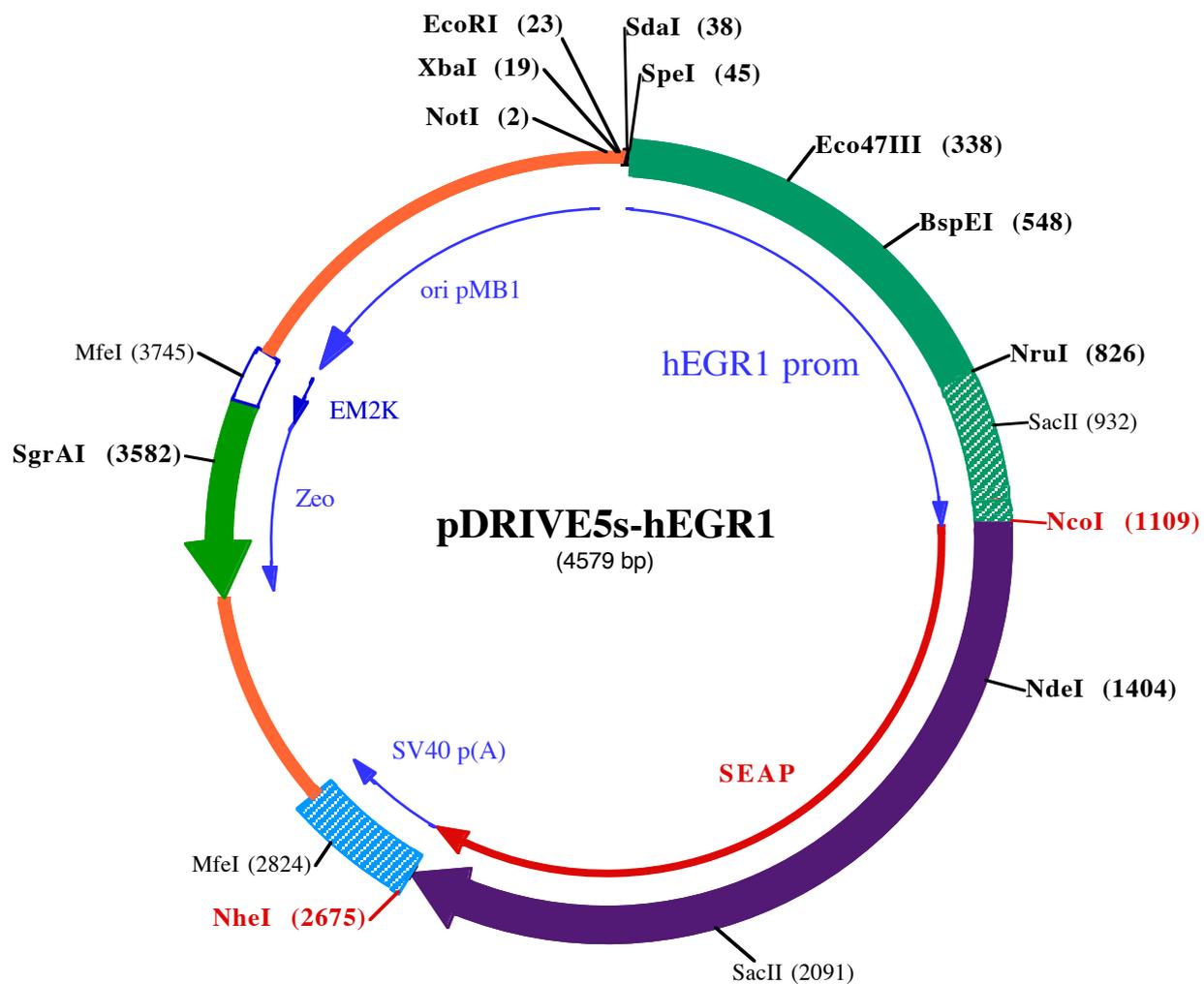
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3301 CAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTGCGCGAGGGCGAACTCCCGCCCCACG
125 •••AspGlnGluAlaValPheHisValCysAsnGlyAlaProAspArgLeuAlaPheGluArgGlyTrpP
3401 GCTGCTCGCCGATCTCGGTTCATGGCCGGCCGGAGGCGTCCCGAAAGTTCTGTGGACACACCTCCGACCCTCGGGCTACAGCTCGTCCAGGCCGCGCAC
100 oGlnGluGlyIleGluThrMetAlaProGlySerAlaAspArgPheAsnThrSerValValGluSerTrpGluAlaTyrLeuGluAspLeuGlyArgVal
SgrAI (3582)
3501 CCACACCCAGGCCAGGGTGTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCTCCGGACCACACCGGCGAAGTCGTCCTCC
67 TrpValTrpAlaLeuThrAsnAspProValValGlnAspGlnValAlaSerIlePheLeuThrValAspAspArgValValGlyAlaPheAspAspGluV
3601 ACGAAGTCCCGGGAGAACCAGCGGTCGGTCCAGAATCGACCGTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCA
33 alPheAspArgSerPheGlyLeuArgAspThrTrpPheGluValAlaGlyAlaValAspArgAlaThrLeuValProValAlaSerThrLeuLysAlaMe
MfeI (3745)
3701 TGATGGCTCCTCCTGTCAGGAGAGGAAAGAGAAGAAGGTTAGTACAATTGCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAAT
0 t
3801 TGTCAAACTAGGGCTGCAGGTTAATTAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCTTGCTGGCGTTTTTCCATAG
3901 GCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGC
4001 TCCCTCGTGCCTCTCCTGTTCGACCCTGCCGTTACCGGATACCTGTCCGCCTTCTCCCTTCGGGAAGCGTGGCGCTTCTCATAGCTCAGCTGTA
4101 GGTATCTCAGTTCGGTGTAGGTCGTTTCGTCCTCAAGCTGGGCTGTGTGCACGAACCCCGTTAGCCCGACCGCTGCGCCTTATCCGGTAACATATCGTCT
4201 TGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGATGTAGGCGGTGCTACAGAGTTCTTG
4301 AAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGAT
4401 CCGGCAAACAACACCAGCTGGTAGCGGTGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTC
4501 TACGGGTCTGACGCTCAGTGAACGAAAACCTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATCA

Fast-Media® Zeo TB

Microwaveable media for selection and propagation of Zeocin™ resistant *E. coli*

Catalog # fas-zn-l

For research use only

Version # 12B09-MM

PRODUCT INFORMATION

Contents:

- 20 individual sealed pouches of Fast-Media® Zeo TB. Each pouch contains the necessary amount of powder for the preparation of **200 ml of Terrific Broth (TB) based liquid medium** supplemented with Zeocin™.

Effective concentration: Zeocin™ 25 µg/ml

Storage and stability:

- Fast-Media® Zeo TB 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® Zeo TB broths are stable for 4 weeks at 4°C, retaining sterility and selective properties.

Quality control:

The high quality and performance of each formulation are tested with *E. coli* K12 derived strains. *E. coli* transformed with a plasmid carrying the Zeocin™ resistance gene are used as positive controls for Fast-Media® Zeo TB.

METHOD

For customer convenience, the following 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 before use.

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.

SPECIAL HANDLING

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

FAST-MEDIA® FEATURES

Fast-Media® offer researchers a quick and convenient way to prepare 200 ml of sterile *E. coli* growth medium in about five minutes using a **microwave** instead of an autoclave.

Fast-Media® is supplied with a choice of antibiotics for selection (see table below), and chromogenic substrates, for the growth or selection of *E. coli* transformant colonies, as well as detection of blue/white colonies. Fast-Media® Base is supplied without selective antibiotics.

Several Fast-Media® are available:

- **Fast-Media® TB**, Terrific Broth based liquid medium
- **Fast-Media® LB**, Lysogeny Broth (LB) based liquid medium
- **Fast-Media® Agar**, LB based solid medium
- **Fast-Media® Agar X-Gal**, LB based solid medium containing IPTG and X-Gal
- **Fast-Media® Agar X-Gluc** LB based solid medium containing X-Gluc.

Fast-Media®	Agar	Agar X-Gal	Agar X-Gluc	LB	TB
Base	X				X
Ampicillin	X	X		X	X
Blasticidin	X	X			X
Hygromycin	X	X			X
Kanamycin	X	X		X	X
Puromycin	X				X
Zeocin™	X	X	X		X

RELATED PRODUCTS

Product	Catalog Code
Fast-Media® Zeo Agar	fas-zn-s
Fast-Media® Zeo Agar X-Gal	fas-zn-x
Fast-Media® Zeo Agar X-Gluc	fas-zn-g

TECHNICAL SUPPORT

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Fast-Media® Zeo Agar

Microwaveable media for selection and propagation of Zeocin™ resistant *E. coli*

Catalog # fas-zn-s

For research use only

Version # 12B10-MM

PRODUCT INFORMATION

Contents:

- 20 individual sealed pouches of Fast-Media® Zeo Agar. Each pouch contains the necessary amount of powder for the preparation of **200 ml of Lysogeny Broth (LB) based solid medium** supplemented with **Zeocin™**. Lysogeny Broth is also known as Luria Broth.

Effective concentration: Zeocin™ 25 µg/ml

Storage and stability:

- Fast-Media® Zeo Agar 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® Zeo Agar broths are stable for 4 weeks at 4°C, retaining sterility and selective properties.

Quality control:

The high quality and performance of each formulation are tested with *E. coli* K12 derived strains. *E. coli* transformed with a plasmid carrying the Zeocin™ resistance gene are used as positive controls for Fast-Media® Zeo Agar.

METHOD

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2. Add 200 ml of distilled or deionized water.
3. Mix thoroughly by swirling the glass bottle or flask.
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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.
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- **Fast-Media® Agar**, LB based solid medium
- **Fast-Media® Agar X-Gal**, LB based solid medium containing IPTG and X-Gal
- **Fast-Media® Agar X-Gluc** LB based solid medium containing X-Gluc.

Fast-Media®	Agar	Agar X-Gal	Agar X-Gluc	LB	TB
Base	X				X
Ampicillin	X	X		X	X
Blasticidin	X	X			X
Hygromycin	X	X			X
Kanamycin	X	X		X	X
Puromycin	X				X
Zeocin™	X	X	X		X

RELATED PRODUCTS

Product	Catalog Code
Fast-Media® Zeo TB	fas-zn-l
Fast-Media® Zeo Agar X-Gal	fas-zn-x
Fast-Media® Zeo Agar X-Gluc	fas-zn-g

TECHNICAL SUPPORT

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