pDRIVE5s-hUbiquitin B

A plasmid with a native ubiquitous human ubiquitin B promoter

Catalog # pDRIVE5s-hubiquitinb

For research use only

Version # 09F17-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), Ø80lacZΔM15, ΔlacX74, rspL (StrA), recA1, endA1 Δdcm ΔsbcC-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 *Bsp*LU11 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 Ubiquitin B promoter

Complete Promoter size: 1090 bp

Specificity: Ubiquitous

Ubiquitin B (Ubi B) is a small highly conserved protein that is abundantly expressed in all eukaryotic cells. Ubi B is involved in the degradation of short-lived regulatory proteins as well as abnormal/mutated proteins and in the processing of major histocompatibility class I-restricted antigens[1]. A 1.1 kb fragment from the ubiquitin B gene was shown to display sustained expression of a transgene *in vitro* and *in vivo*. Twenty days post-injection, the levels of expression were similar to those obtained with the CMV promoter and were higher 35 days post-injection[2].

1. Ciechanover A. and Schwartz AL. 1998. The ubiquitin-proteasome pathway: the complexity and myriad functions of proteins death. Proc Natl Acad Sci USA 95(6):2727-30.
2. Yew NS. *et al.* 2001. High and sustained transgene expression in vivo from plasmid vectors containing a hybrid ubiquitin promoter. Mol Ther. 4(1):75-82.

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.*

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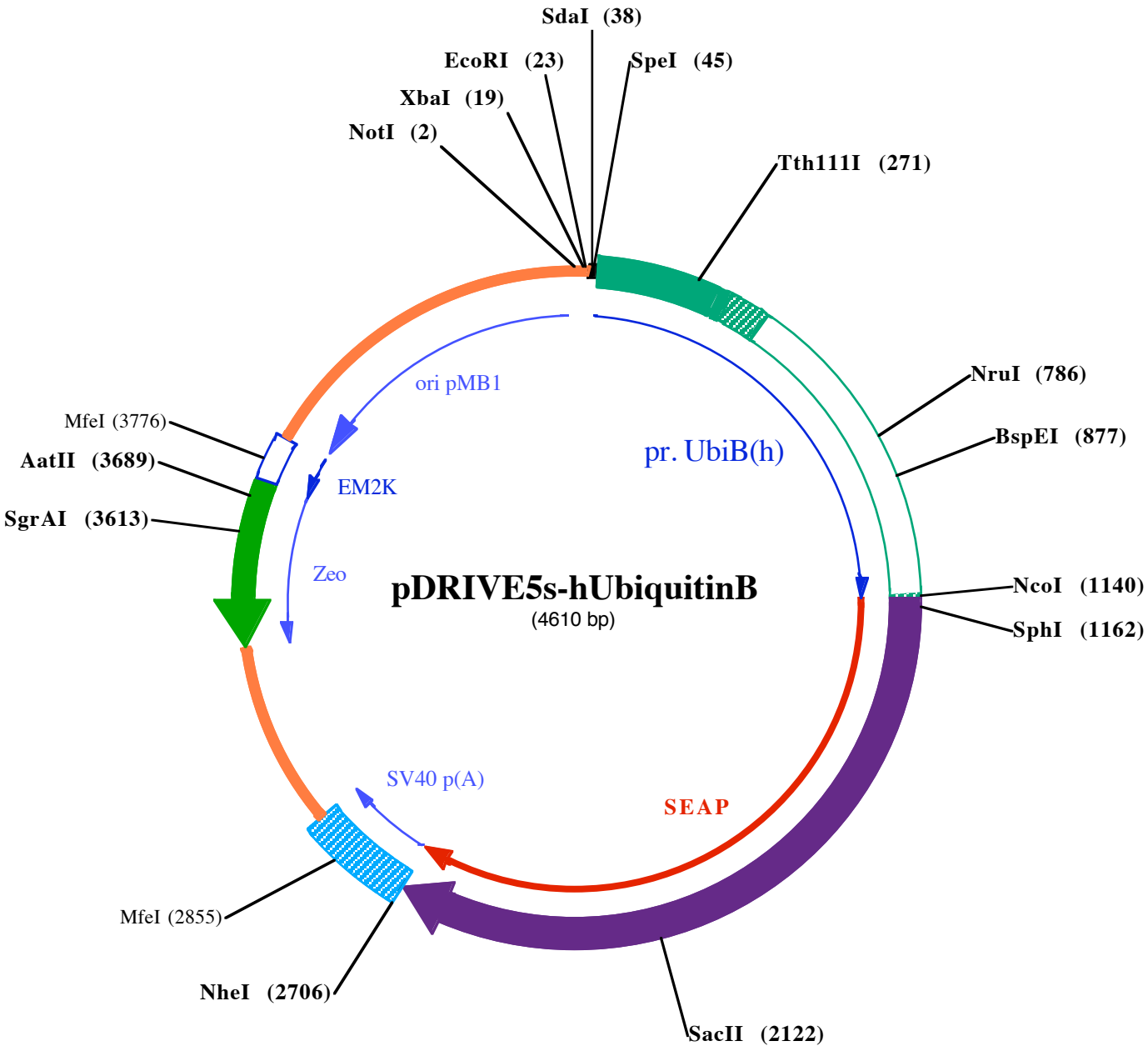
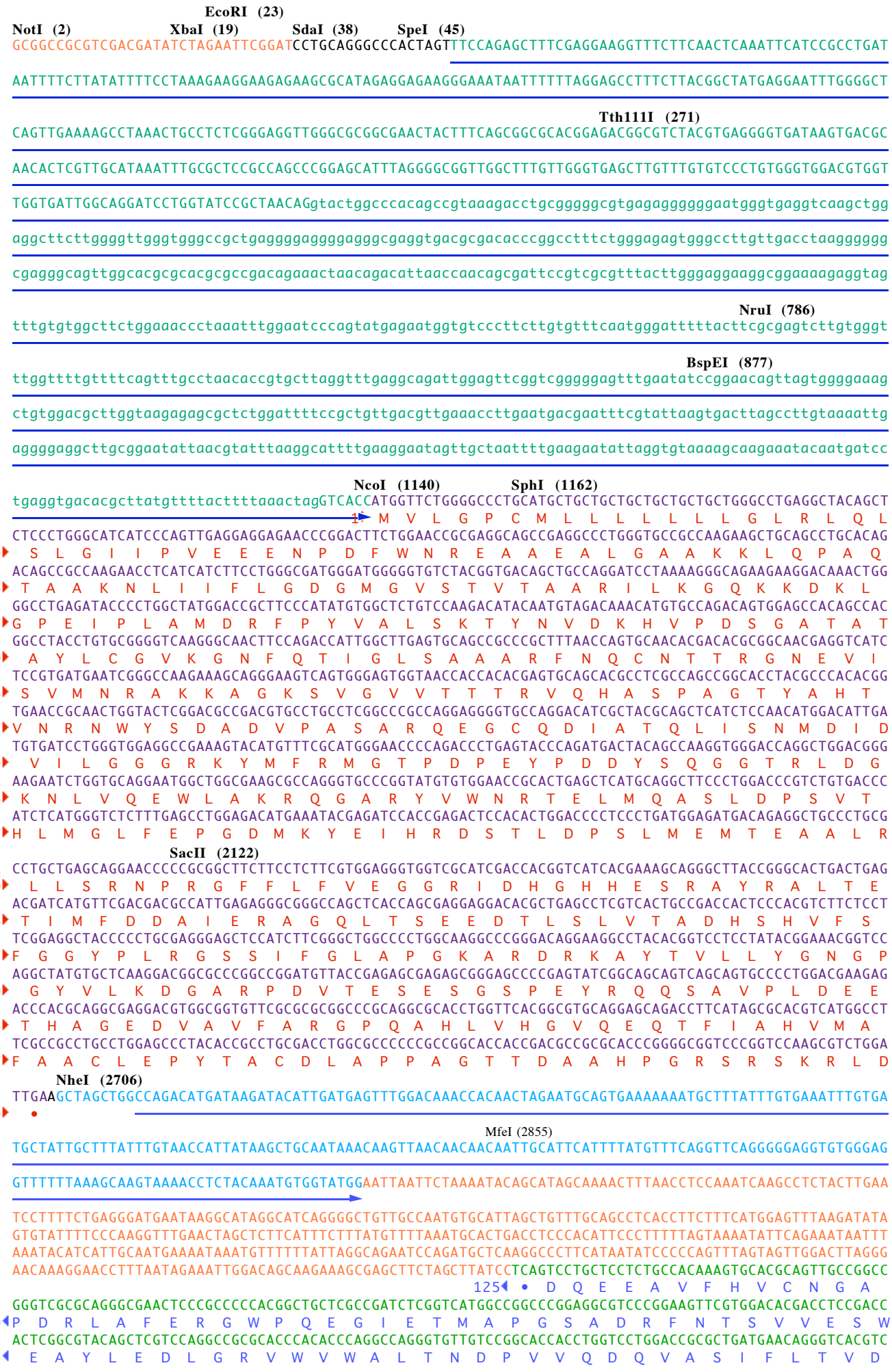

SdaI (38)
EcoRI (23)
SpeI (45)
XbaI (19)
NotI (2)
Tth111I (271)
NruI (786)
BspEI (877)
NcoI (1140)
SphI (1162)
SacII (2122)
MfeI (2855)
NheI (2706)
MfeI (3776)
AatII (3689)
SgrAI (3613)
ori pMB1
pr. UbiB(h)
EM2K
Zeo
pDRIVE5s-hUbiquitinB
(4610 bp)
SV40 p(A)
SEAP

InvivoGen


EcoRI (23)
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SacII (2122)
NheI (2706)
MfeI (2855)


SgrAI (3613)
AatII (3689)
3601 GTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGGCGACGTCGCGCGCGGTG
44 D R V V G A F D D E V F D R S F G L R D T W F E V A G A V D R A T
MfeI (3776)
3701 AGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCCTGTCAGGAGAGGAAAGAGAAGAAGGTTAGTACAATTGCTATAGTGAGTTGTATTAT
10 L V P V A S T L K A M
3801 ACTATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTA
3901 AAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACT
4001 ATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGA
4101 AGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCG
4201 ACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGC
4301 GAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTT
4401 ACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAA
4501 AAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAAC
4601 ATTTAAATCA

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 # 09G27-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 at least one year at room temperature.
When properly prepared, **Fast-Media®** plates or broths are stable several 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.

	Agar	X-Gal	TB
Base	√		√
Ampicillin	√	√	√
Blasticidin	√	√	√
Hygromycin	√	√	√
Kanamycin	√	√	√
Puromycin	√		√
Zeocin™	√	√	√

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*.

TECHNICAL SUPPORT
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