pDRIVE5SEAP-meIF4A1

A plasmid with a native ubiquitous murine eukaryotic initiation factor 4A1 promoter
Catalog # pDRIVE5s-meif4a1

For research use only
Version # 09H19-MM

PRODUCT INFORMATION
Content:
- 1 disk of lyophilized GT116 *E. coli* bacteria transformed by a pDRIVE5-SEAP 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
pDRIVE5-SEAP is an expression plasmid containing a native or composite promoter of interest. **pDRIVE5-SEAP** 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 pDRIVE5-SEAP 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
Murine eukaryotic initiation factor 4A1 promoter
Complete Promoter size: 483 bp
Specificity: Ubiquitous

The eIF-4A1 gene encoding eukaryotic initiation factor 4A is ubiquitously expressed in eukaryotic cells. The minimal promoter of the eIF-4A1 gene consists of approximately 300 bp, 80% of which are identical with those in mouse eIF-4A1 promoter. The minimal promoter contains TATA and CAAT motifs and consensus sequences binding to SP1 and AP2[1]. The eIF-4A1 promoter is a versatile promoter giving high-levels of expression. In some cell lines, such as macrophages, levels of expression are higher than that obtained with the CMV promoter[2].

1. Kukimoto I. et al. 1997. Characterization of the cloned promoter of the human initiation factor 4AI gene. Biochem Biophys Res Commun 233(3):844-7.
2. Quinn CM. et al. 1999. The human eukaryotic initiation factor 4AI gene (EIF4A1) contains multiple regulatory elements that direct high-level reporter gene expression in mammalian cell lines. Genomics 62(3):468-76.

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 pDRIVE5-SEAP 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 pDRIVE5-SEAP 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.

NotI (2)
XbaI (19)
EcoRI (23)
SdaI (38)
SpeI (45)
AflII (272)
NcoI (531)

NdeI (826)

SacII (1513)

NheI (2097)
MfeI (2246)

SV40 p(A)

SEAP

pDRIVE5s-meIF4A1
(4001 bp)

eIF-4AI(m) prom

ori pMB1

EM2K

Zeo

MfeI (3167)
SgrAI (3004)

InvivoGen
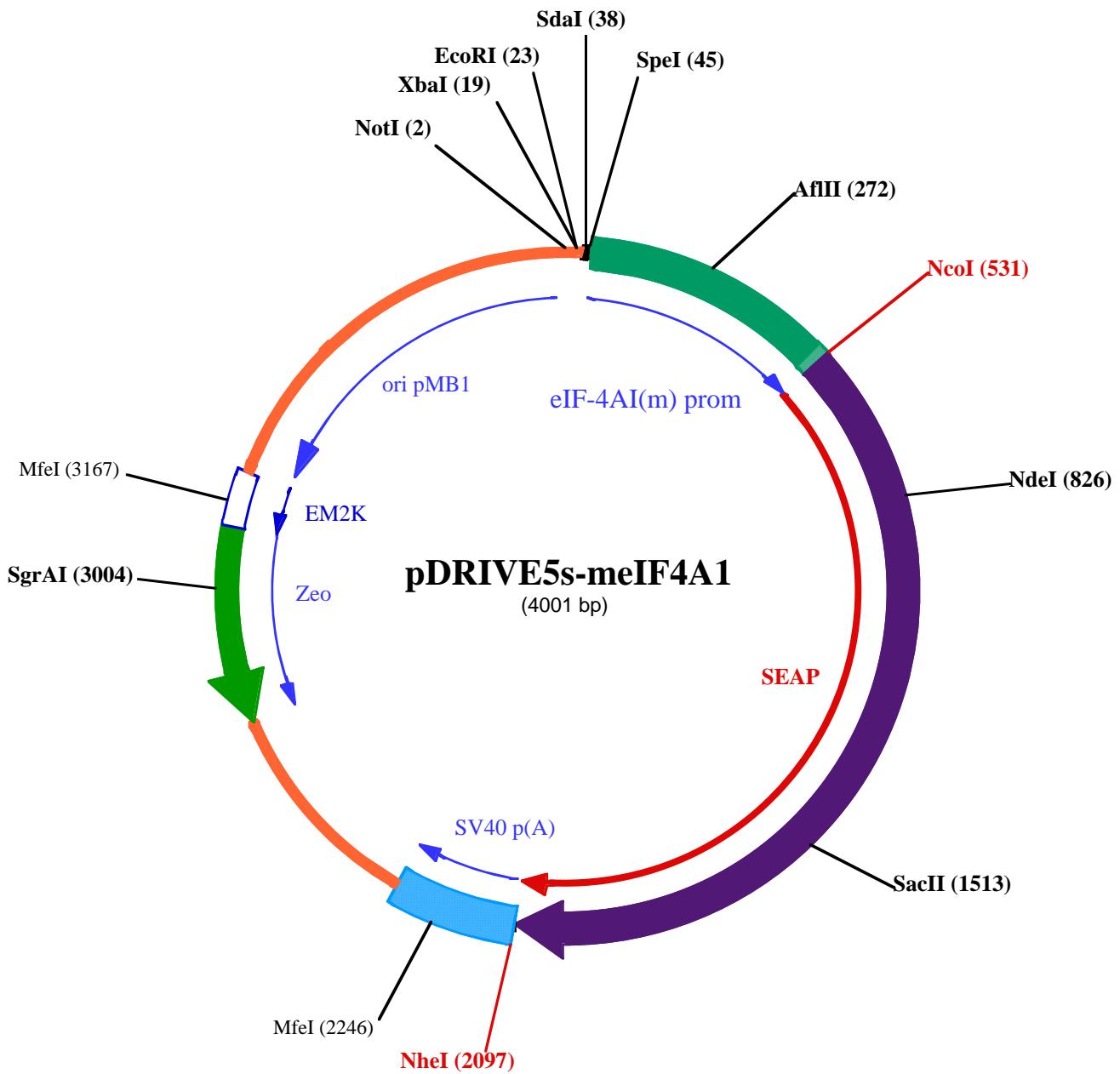
EcoRI (23)

NotI (2)   XbaI (19)   SdaI (38)   SpeI (45)

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1    GCGGCCGCGTCGACGATATCTAGAATTCGGATCCTGCAGGGCCCACTAGTCGCTACAATATTTTCCTGAACGGAAGAATAAATAAAACTTGTCCTGTAAA

101  GAAAACCCAGGTAAAGGAAAGTGGCAGTCCAGACTGCCCGGAAGTTCCTGGAGGCTAAGGCCTCACCCCCGTCGCTTGATAGGACCTGCTGAGCCACATG
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AflII (272)

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201  ACTAAGGCACGATCGCCTCCGCACGTGTAAAGGTGCTGGGTTCCAAGATGGCTGCCCCGCCGCGAGGCCCGACTTAAGTATGTCACTTCCGCACCAGCGA

301  GAAAGGCGGACCCTTCAGCCAATGAGGCCATAGGGCGGGGCTAGGCCATGATGGGCTTTCAAACTACCCAATAGGGCGTCCGAACTAAAGCGCCTACAAA

401  GTAACGTCACGTCGAGTTGCAGAGCGCCGGCAGGCGGGGCAGAGGTGGCCAAGCCAATGCGATGGCTGGGGCGGGGTCGGACGCTCTATAAGTTGTCGAT
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NcoI (531)

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501  AGGCGGGCACTCCGCCCTAGATTCTAAGGACCATGGTTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCTGCTGGGCCTGAGGCTACAGCTCTCCCTGGG
                                      1  M  V  L  G  P  C  M  L  L  L  L  L  L  L  G  L  R  L  Q  L  S  L  G

601  CATCATCCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGCGAGGCAGCCGAGGCCCTGGGTGCCGCCAAGAAGCTGCAGCCTGCACAGACAGCCGCC
     23  I  I  P  V  E  E  N  P  D  F  W  N  R  E  A  A  E  A  L  G  A  A  K  K  L  Q  P  A  Q  T  A  A

701  AAGAACCTCATCATCTTCCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAAACTGGGGCCTGAGA
     57  K  N  L  I  I  F  L  G  D  G  M  G  V  S  T  V  T  A  A  R  I  L  K  G  Q  K  K  D  K  L  G  P  E
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NdeI (826)

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801  TACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACATGTGCCAGACAGTGGAGCCACAGCCACGGCCTACCT
     90  I  P  L  A  M  D  R  F  P  Y  V  A  L  S  K  T  Y  N  V  D  K  H  V  P  D  S  G  A  T  A  T  A  Y  L

901  GTGCGGGGTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCGCCCGCTTTAACCAGTGCAACACGACACGCGGCAACGAGGTCATCTCCGTGATG
     123  C  G  V  K  G  N  F  Q  T  I  G  L  S  A  A  A  R  F  N  Q  C  N  T  T  R  G  N  E  V  I  S  V  M

1001 AATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACACGAGTGCAGCACGCCTCGCCAGCCGGCACCTACGCCCACACGGTGAACCGCA
     157  N  R  A  K  K  A  G  K  S  V  G  V  V  T  T  T  R  V  Q  H  A  S  P  A  G  T  Y  A  H  T  V  N  R

1101 ACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCCAGGAGGGGTGCCAGGACATCGCTACGCAGCTCATCTCCAACATGGACATTGATGTGATCCT
     190 N  W  Y  S  D  A  D  V  P  A  S  A  R  Q  E  G  C  Q  D  I  A  T  Q  L  I  S  N  M  D  I  D  V  I  L

1201 GGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCCAGACCCTGAGTACCCAGATGACTACAGCCAAGGTGGGACCAGGCTGGACGGGAAGAATCTG
     223  G  G  G  R  K  Y  M  F  R  M  G  T  P  D  P  E  Y  P  D  D  Y  S  Q  G  G  T  R  L  D  G  K  N  L

1301 GTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCCGGTATGTGTGGAACCGCACTGAGCTCATGCAGGCTTCCCTGGACCCGTCTGTGACCCATCTCATGG
     257  V  Q  E  W  L  A  K  R  Q  G  A  R  Y  V  W  N  R  T  E  L  M  Q  A  S  L  D  P  S  V  T  H  L  M

1401 GTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCCTGCTGAG
     290 G  L  F  E  P  G  D  M  K  Y  E  I  H  R  D  S  T  L  D  P  S  L  M  E  M  T  E  A  A  L  R  L  L  S
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SacII (1513)

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1501 CAGGAACCCCCGCGGCTTCTTCCTCTTCGTGGAGGGTGGTCGCATCGACCACGGTCATCACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATG
     323  R  N  P  R  G  F  F  L  F  V  E  G  G  R  I  D  H  G  H  H  E  S  R  A  Y  R  A  L  T  E  T  I  M

1601 TTCGACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACACGCTGAGCCTCGTCACTGCCGACCACTCCCACGTCTTCTCCTTCGGAGGCT
     357  F  D  D  A  I  E  R  A  G  Q  L  T  S  E  E  D  T  L  S  L  V  T  A  D  H  S  H  V  F  S  F  G  G

1701 ACCCCCTGCGAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAGGCCCGGGACAGGAAGGCCTACACGGTCCTCCTATACGGAAACGGTCCAGGCTATGT
     390  Y  P  L  R  G  S  S  I  F  G  L  A  P  G  K  A  R  D  R  K  A  Y  T  V  L  L  Y  G  N  G  P  G  Y  V

1801 GCTCAAGGACGGCGCCCGGCCGGATGTTACCGAGAGCGAGAGCGGGAGCCCCGAGTATCGGCAGCAGTCAGCAGTGCCCCTGGACGAAGAGACCCACGCA
     423  L  K  D  G  A  R  P  D  V  T  E  S  E  S  G  S  P  E  Y  R  Q  Q  S  A  V  P  L  D  E  E  T  H  A

1901 GGCGAGGATGTGGCGGTGTTCGCGCGCGGCCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGACCTTCATAGCGCACGTCATGGCCTTCGCCGCT
     457  G  E  D  V  A  V  F  A  R  G  P  Q  A  H  L  V  H  G  V  Q  E  Q  T  F  I  A  H  V  M  A  F  A  A
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NheI (20

```
2001 GCCTGGAGCCCTACACCGCCTGCGACCTGGCGCCCCCGCCGGCACCACCGACGCCGCGCACCCGGGGCGGTCCCGGTCCAAGCGTCTGGATTGAAGCTA
     490 C  L  E  P  Y  T  A  C  D  L  A  P  P  A  G  T  T  D  A  A  H  P  G  R  S  R  S  K  R  L  D  •

2101 GCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGC
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MfeI (2246)

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2201 TTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAGGTTCAGGGGGAGGTGTGGGAGGTTTTTTAA

2301 AGCAAGTAAAACCTCTACAAATGTGGTATGGAATTAATTCTAAAATACAGCATAGCAAAACTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCT

2401 GAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTT

2501 CCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATC

2601 ATTGCAATGAAAATAAATGTTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGA

2701 ACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGGTCGCGC
                                                        125 •  D  Q  E  E  A  V  F  H  V  C  N  G  A  P  D  R

2801 AGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGT
     107 L  A  F  E  R  G  W  P  Q  E  G  I  E  T  M  A  P  G  S  A  D  R  F  N  T  S  V  V  E  S  W  E  A  Y

2901 ACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGAC
     74  L  E  D  L  G  R  V  W  V  W  A  L  T  N  D  P  V  V  Q  D  Q  V  A  S  I  F  L  T  V  D  D  R  V
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SgrAI (3004)

```
3001 CACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCTCCGCGCGACGTCGCGCGCGGTGAGCACCGGA
     41  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  L  V  P
```

MfeI (3167)

```
3101 ACGGCACTGGTCAACTTGGCCATGATGGCTCCTCCTGTCAGGAGAGGAAAGAGAAGAAGGTTAGTACAATTGCTATAGTGAGTTGTATTATACTATGCAG
     7 V  A  S  T  L  K  A  M  ◄

3201 ATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCG

3301 CGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATA

3401 CCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCG
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3501  CTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCG

3601  CCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGT

3701  AGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGA

3801  AAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTC

3901  AAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATC

4001  A
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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*.

InvivoGen

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