

pDRIVE5s-hCOX-2

A plasmid with a native tumor-specific human cyclo-oxygenase 2 isoform promoter

Catalog # pdrive5s-hcox2

For research use only

Version # 11G05-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, 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

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' restriction site is Spe I is compatible with Avr II, Nhe I and Xba I. The 3' restriction site is Bsp H I.
- **Compare the activity of different promoters** in transient transfection experiments. Each pDRIVE 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 (Bsp HI and Nhe I) for easy replacement with a different gene of interest.

PROMOTER CHARACTERISTICS

Human cyclo-oxygenase 2 isoform (hCOX-2)

Complete Promoter size: 1568 bp
Specificity : Tumor

COX-2 is an inducible isoform of cyclooxygenase. Expression of COX-2 is undetectable under physiological conditions but up-regulated in many malignant tumors. COX-2, which is a PKC-dependent gene, is important for the genesis of cancer. COX-2 promoter contains a cAMP response element and sites for AP-2 and NF-κB that are both PKC-responsive cis-elements. COX-2 promoter has been used to target gene expression in pancreatic¹, gastrointestinal² and ovarian cancers. Similar levels of expression of luciferase and HSVtk genes were obtained with COX-2 and CMV promoters.

1. **Wesseling JG et al. 2001.** Midkine and cyclooxygenase-2 promoters are promising for adenoviral vector gene delivery of pancreatic carcinoma. *Cancer Gene Ther* 8(12):990-6.
2. **Yamamoto M et al. 2001.** Characterization of the cyclooxygenase-2 promoter in an adenoviral vector and its application for the mitigation of toxicity in suicide gene therapy of gastrointestinal cancers. *Mol Ther.* 3(3):385-94.

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 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.

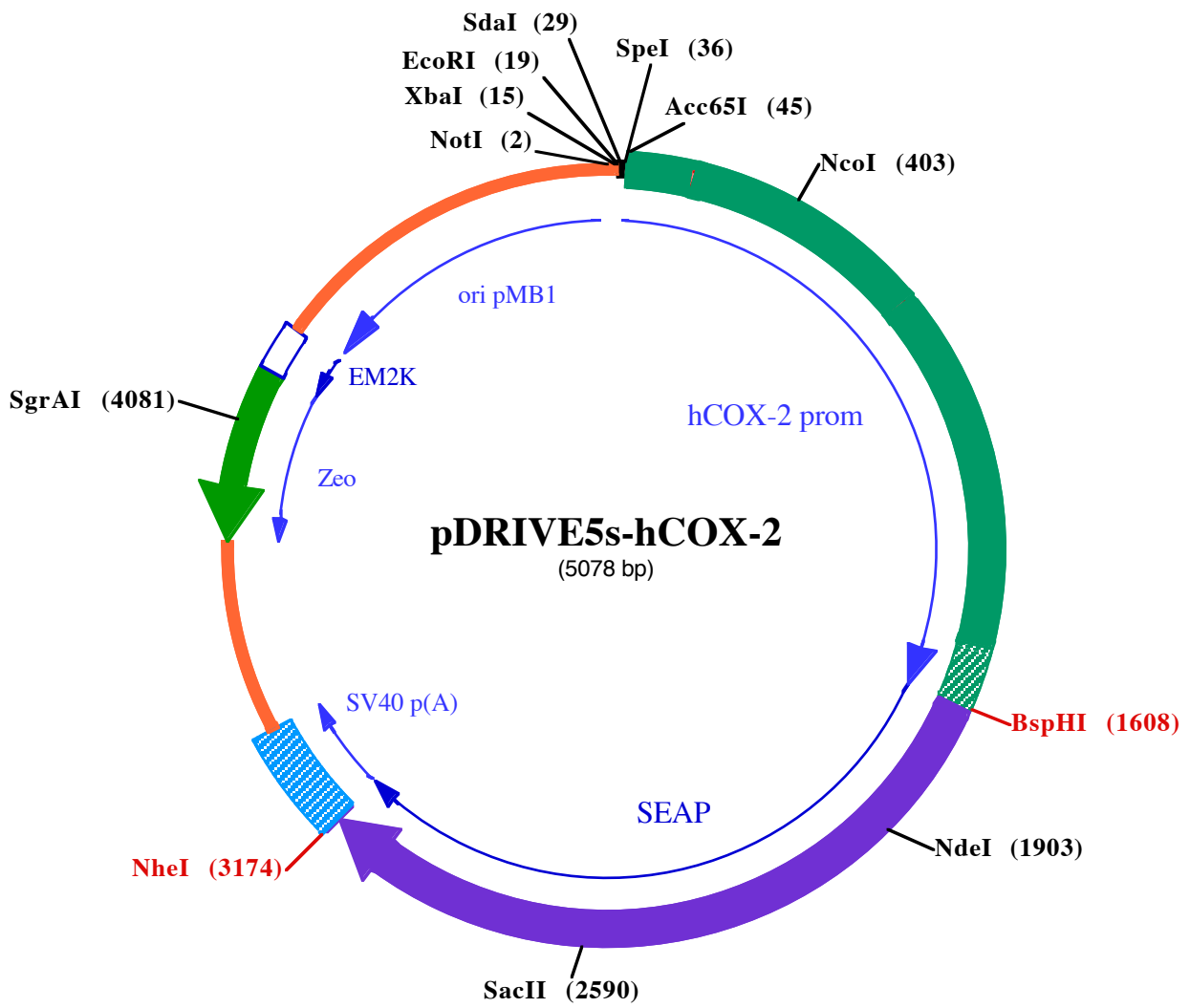
Note: Do not reheat solidified Fast-Media® as the antibiotic will be permanently destroyed by the procedure.

TECHNICAL SUPPORT

Toll free (US): 888-457-5873
Outside US: (+1) 858-457-5873
Europe: +33 562-71-69-39
E-mail: info@invivogen.com
Website: www.invivogen.com



3950 Sorrento Valley Blvd. Suite 100
San Diego, CA 92121 - USA



EcoRI (19)

NotI (2) XbaI (15) SdaI (29) SpeI (36) Acc65I (45)

1 CGCGCCGCTATGCATCTAGAATTCCTGCAGGGCCCACTAGTTGAGGTACCTGGTGTAGTTTTATTTTCAGGTTTTATGCTGTCATTTTCTGTAATGCTAA
101 GGACTTAGGACATAACTGAATTTTCTATTTTCCACTTCTTTTCTGGTGTGTGTATATATATATATATATATATACACACACACATGTACATATATATATT
201 TTTTAGTATCTCACCTCACATGCTCCTCCTGAGCACTACCCATGATAGATGTTAAACAAAAGCAAAGATGAAATTCCAACGTCAAAATCTCCCTTCC
301 ATCTAATTAATTCCTCATCAACTATGTTCCAAAACGAGAATAGAAAATTAGCCCAATAAGCCCAGGCAACTGAAAAGTAAATGCTATGTTGTACTTTG
NcoI (403)
401 ATCCATGGTCACAACCTATAATCTTGAAAAGTGGACAGAAAAGACAAAAGAGTGAACCTTAAAACCTCGAATTTATTTTACCAGTATCTCCTATGAAGGG
501 CTAGTAACCAAATAATCCACGCATCAGGGAGAGAAAATGCCTTAAGGCATACGTTTTGGACATTTAGCGTCCCTGCAAATTTCTGGCCATCGCCGCTTCT
601 TTGTCATCAGAAGCGAGGAAACTTATATTGGTGACCCGTGGAGCTCACATTAACCTATTACAGGGTAACTGCTTAGGACCAAGTATTATGAGGAGAATT
701 TACCTTTCCCTCCTCTTTTCAAGAAACAAGGAGGGGTGAAGGTACGGAGAACAGTATTTCTTCTGTTGAAAGCAACTTAGCTACAAAGATAAATTAC
801 AGCTATGTACTGAAGGTAGCTATTTTATTCCACAAAATAAGAGTTTTTAAAAAGCTATGTATGTATGTGCTGCATATAGAGCAGATATACAGCCTAT
901 TAAGCGTCGCTACTAAAACATAAAACATGTCAGCCTTTCTAACCTTACTCGCCCCAGTCTGTCCCGACGTGACTTCTCGACCCTTAAAGACGTACAG
1001 ACCAGACACGGCGGGCGGGGAGAGGGGATTCCCTGCGCCCCCGACCTCAGGGCCGCTCAGATTCTGGAGAGGAAGCCAAGTGTCTTCTGCCCT
1101 CCCCCGGTATCCCATCAAGCGCATCAGTCCAGAAGCTGCTCGGAAGCGCTCGGGCAAAGACTGCGAAGAAGAAAAGACATCTGGCGGAAACCTGTGC
1201 GCCTGGGGCGGTGGAACCTCGGGGAGGAGGGAGGGATCAGACAGGAGAGTGGGGACTACCCCTCTGCTCCCAAATTTGGGCGAGCTTCTGGGTTCCG
1301 ATTTTCTCATTTCGGTGGGTA AAAAACCTGCCCCACCGGGCTTACGCAATTTTTTAAAGGGGAGAGGAGGAAAAATTTGTGGGGGTACGAAAAGGC
1401 GGAAAGAACAGTCATTTTCGTCACATGGGCTTGGTTTTTTCAGTCTTATAAAAAGGAAGTTCTCTCGGTTAGCGACCAATTGTCATACGACTTGCAAGTGA
1501 CGTCAGGAGCACGTCAGGAACCTCCTCAGCAGCGCTCCTCAGTCCACAGCCAGACGCCCTCAGACAGCAAAGCCTACCCCGCGCCGCGCCCTGCC

BspHI (1608)

1601 GCGCTGTATGATTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCTGCTGGGCTGAGGCTACAGCTCTCCCTGGGCATCATCCAGTTGAGGAGGAGA
M I L G P C M L L L L L L L L G L R L Q L S L G I I P V E E E
1701 ACCCGGACTTCTGGAACCGCGAGGACCGGAGGCCCTGGGTGCCGCAAGAAGCTGCAGCCTGCACAGACAGCCGCAAGAACCTCATCATTTCTGGG
31 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 K N L I I F L G
1801 CGATGGGATGGGGTGTCTACGGTACAGCTGCCAGGATCCTAAAAGGGCAGAAGAAGGACAAACTGGGGCTGAGATACCCCTGGCTATGGACCGCTC
64 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 I P L A M D R F

NdeI (1903)

1901 CCATATGTGGCTCTGTCCAAGACATAACAATGTAGACAAAACATGTGCCAGACAGTGGAGCCACAGCCACGGCTACCTGTGCGGGGTCAAGGGCAACTTCC
98 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 C G V K G N F
2001 AGACCATTGGCTTGAAGTGCAGCCGCCCGCTTAAACAGTGAACACGACACGCGGCAACGAGGTCATCTCCGTGATGAATCGGGCAAGAAGCAGGGAA
131 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 N R A K K A G K
2101 GTCAGTGGGAGTGGTAACCAACACAGTGCAGCAGCCTCGCCAGCCGGCACCTACGCCACACGGTGAACCGCAACTGGTACTCGGACGCCGACGTV
164 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 N W Y S D A D V
2201 CCTGCCTCGGCCCGCAGGAGGGGTGCCAGGACATCGCTACGAGCTCATCTCCAACATGGACATTGATGTGATCCTGGGTGGAGGCCGAAAGTACATGT
198 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 G G G R K Y M
2301 TTCGCATGGGAACCCAGACCTGAGTACCCAGATGACTACAGCAAGGTGGGACCAGGCTGGACGGGAAGAACTGGTGCAGGAATGGCTGGCGAAGCG
231 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 V Q E W L A K R
2401 CCAGGGTGCCTGGTGTGTGGAACCGCACTGAGCTCATGAGGCTTCCCTGGACCCGCTGTGACCCATCTCATGGGTCTCTTTGAGCCTGGAGACATG
264 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 G L F E P G D M

SacII (2590)

2501 AAATACGAGATCCACCGAGACTCCACACTGGACCCTCCCTGATGGAGATGACAGAGGCTGCCCTGCGCCTGCTGAGCAGGAACCCCGCGGCTTCTTCC
298 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 R N P R G F F
2601 TCTTCGTGGAGGGTGGTGCATCGACCAGGTCATCACGAAAGCAGGGCTTACCGGGCACTGACTGAGACGATCATGTTTCGACGACGCCATTGAGAGGGC
331 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 F D D A I E R A
2701 GGGCAGCTCACCAGCGAGGAGGACACGCTGAGCCTCGTCACTGCCGACCACTCCACGTTCTCTCTTCGGAGGCTACCCCTGCGAGGGAGCTCCATC
364 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 Y P L R G S S I
2801 TTCGGGCTGGCCCTGGCAAGGCCCGGGACAGGAAGCCATACCGTCTCTATACGAAAACGGTCCAGGCTATGTCTCAAGGACGGCCGCGCCGCGG
398 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 L K D G A R P
2901 ATGTTACCAGAGCGAGAGCGGGAGCCCGAGTATCGGCAGCAGTCAAGTGGCCCTGGACGAAGAGACCCACGAGGCGAGGACGTGGCGGTGTTCCG
431 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 G E D V A V F A
3001 GCGCGGCCCGCAGGCGCACCTGTTTACGGCGTGCAGGAGCAGACCTTATAGCGCACGTCATGGCCTTTCGCGCCTGCCTGGAGCCCTACACCGCCTGC
464 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 C L E P Y T A C

NheI (3174)

3101 GACCTGGCGCCCCCGCGGCACCACCGACCGCGCACCCGGGGCGGTCCCGGTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATAC
498 D L A P P A G T T D A A H P G R S R S K R L D
3201 ATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCT
3301 GCAATAAACAAAGTTAAACAACAACATTGCATTTATGTTTTCAGGTTTCAAGGGGAGGTGGGGAGGTTTTTAAAGCAAGTAAAACCTCTACAAATG
3401 TGGTATGGAAATTAATTCTAAATACAGCATAGCAAACCTTAACTCCAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCA

3501 TCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAGTTTAAGATATAGTGTATTTTCCAAGGTTTGAAGTCTCTTC
3601 ATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAATATTAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTT
3701 TTATTAGGCAGAAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAGGAACCTTTAATAGAAATTGGACAGC
3801 AAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTTGCCACAAAGTGCACGCAAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCACGG
125 • D Q E E A V F H V C N G A P D R L A F E R G W P
3901 CTGCTCGCCGATCTCGGTATGGCCGGCCGGAGGCGTCCCGGAAGTTCTGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACC
100 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 L E D L G R V
SgrAI (4081)
4001 CACACCCAGGCCAGGGTGTGTCCGGCACCACTGGTCTGGACCGCGTGTATGAACAGGGTCACGTCGTCCCGGACCACCCGGCGAAGTCTCTCCA
66 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 V G A F D D E V
4101 CGAAGTCCCGGGAGAACCAGCCGGTCCGAGTCCAGAACTGACCGCTCCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCAT
33 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 V A S T L K A M
4201 GATGGTCTCTCTGTCAGGAGAGGAAAGAGAAGAAGGTTAGTACAATTGCTATAGTGAGTTGATTATACTATGCAGATATACTATGCCAATGATTAATT
←
4301 GTCAA ACTAGGGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTCTGCGCTTTTCCATAGG
←
4401 CTCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTCCCCCTGGAAGCT
4501 CCCTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCCTTCTCCCTTCGGGAAGCGTGCGCTTCTCATAGCTCACGCTGTAG
4601 GTATCTCAGTTTCGGTGTAGGTCGTTCCGCTCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCCAGCCGCTGCGCTTATCCGGTAACTATCGTCTT
4701 GAGTCAACCCGGTAAGACAGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGA
4801 AGTGGTGGCCTAACTACGGTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATC
4901 CGGCAAAACAACCCGCTGGTAGCGGTGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCT
5001 ACGGGTCTGACGCTCAGTGAACGAAACTCACGTTAAGGGATTTTGGTATGGCTAGTTAATTAACATTTAAATCA

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 # 10G07-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 2 years at room temperature.

When properly prepared, **Fast-Media**® plates or broths are stable for 4 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, Blastidicin 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

Toll free (US): 888-457-5873
Outside US: (+1) 858-457-5873
Europe: +33 562-71-69-39
E-mail: info@invivogen.com
Website: www.invivogen.com


3950 Sorrento Valley Blvd. Suite 100
San Diego, CA 92121 - USA