pDRIVE5s-hCD14

A plasmid with a native tissue specific human CD14 promoter

Catalog # pdrive5s-hcd14

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, 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 *BspL*U11 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 CD14 promoter

Complete Promoter size: 613 bp

Specificity: Monocytic cells

CD14 is a receptor for bacterial lipopolysaccharide expressed on the surface of myeloid-derived cells, and is strongly upregulated during monocytic cell differentiation. Monocyte-specific expression of CD14 is regulated at the transcriptional level. The CD14 promoter contains a C/EBP[1] and Sp1[2] site that are critical for tissue-specific regulation of CD14 gene expression.

1. Pan Z. et al. 1999. CCAAT/enhancer-binding protein activates the CD14 promoter and mediates transforming growth factor beta signaling in monocyte development. J Biol Chem 274(33):23242-8. **2. Park S. et al. 2002.** Synergistic interaction of MEF2D and Sp1 in activation of the CD14 promoter. Mol Immunol 39(1-2):25.

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
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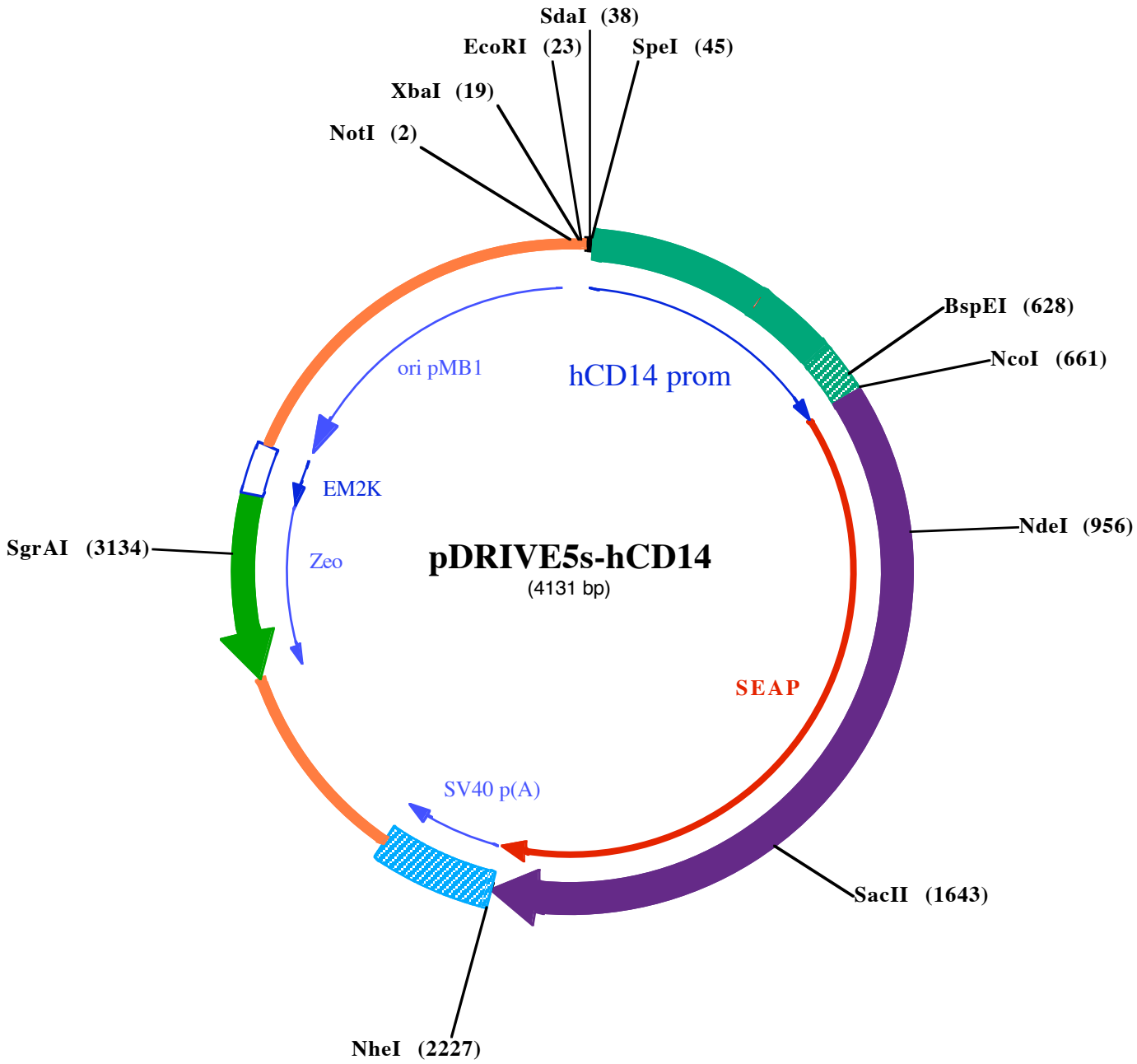
SdaI (38)
EcoRI (23)
SpeI (45)
XbaI (19)
NotI (2)
BspEI (628)
NcoI (661)
ori pMB1
hCD14 prom
EM2K
NdeI (956)
SgrAI (3134)
Zeo
pDRIVE5s-hCD14
(4131 bp)
SEAP
SV40 p(A)
SacII (1643)
NheI (2227)

InvivoGen

EcoRI (23)
NotI (2) XbaI (19) SdaI (38) SpeI (45)
1 GCGGCCGCGTCGACGATATCTAGAATTCGGATCCTGCAGGGCCCACTAGTGCCAACAGATGAGGTTCACAATCTCTTCCACAAAACATGCAGTTAAATAT

101 CTGAGGATATTCAGGGACTTGGATTTGGTGGCAGGAGATCAACATAAACCAAGACAAGGAAGAAGTCAAAGAAATGAATCAAGTAGATTCTCTGGGATAT

201 AAGGTAGGGGGATTGGGGGGTTGGATAGTGCAGAGTATGGTACTGGCCTAAGGCACTGAGGATCATCCTTTTCCCACACCCACCAGAGAAGGCTTAGGCT

301 CCCGAGTCAACAGGGCATTCACCGCCTGGGGCGCCTGAGTCATCAGGACACTGCCAGGAGACACAGAACCCTAGATGCCCTGCAGAATCCTTCCTGTTAC

401 GGTCCCCCTCCCTGAAACATCCTTCATTGCAATATTTCCAGGAAAGGAAGGGGGCTGGCTCGGAGGAAGAGAGGTGGGGAGGTGATCAGGGTTCACAGAG

501 GAGGGAACTGAATGACATCCCAGGATTACATAAACTGTCAGAGGCAGCCGAAGAGTTCACAAGTGTGAAGCCTGGAAGCCGGCGGGTGCCGCTGTGTAGG

BspEI (628) NcoI (661)
601 AAAGAAGCTAAAGCACTTCCAGAGCCTGTCCGGAGCTCAGAGGTTCGGAAGACTTATCGACCATGGTTCTGGGGCCCTGCATGCTGCTGCTGCTGCTGCT
1 M V L G P C M L L L L L L

701 GCTGGGCCTGAGGCTACAGCTCTCCCTGGGCATCATCCCAGTTGAGGAGGAGAACCCGGACTTCTGGAACCGCGAGGCAGCCGAGGCCCTGGGTGCCGCC
13 L G L R L Q L S L G I I P V E E E N P D F W N R E A A E A L G A A

801 AAGAAGCTGCAGCCTGCACAGACAGCCGCCAAGAACCTCATCATCTTCCTGGGCGATGGGATGGGGGTGTCTACGGTGACAGCTGCCAGGATCCTAAAAG
47 K K L Q P A Q T A A K N L I I F L G D G M G V S T V T A A R I L K

NdeI (956)
901 GGCAGAAGAAGGACAAACTGGGGCCTGAGATACCCCTGGCTATGGACCGCTTCCCATATGTGGCTCTGTCCAAGACATACAATGTAGACAAACATGTGCC
80 G Q K K D K L G P E I P L A M D R F P Y V A L S K T Y N V D K H V P

1001 AGACAGTGGAGCCACAGCCACGGCCTACCTGTGCGGGGTCAAGGGCAACTTCCAGACCATTGGCTTGAGTGCAGCCGCCCGCTTTAACCAGTGCAACACG
113 D S G A T A T A Y L C G V K G N F Q T I G L S A A A R F N Q C N T

1101 ACACGCGGCAACGAGGTCATCTCCGTGATGAATCGGGCCAAGAAAGCAGGGAAGTCAGTGGGAGTGGTAACCACCACACGAGTGCAGCACGCCTCGCCAG
147 T R G N E V I S V M N R A K K A G K S V G V V T T T R V Q H A S P

1201 CCGGCACCTACGCCCACACGGTGAACCGCAACTGGTACTCGGACGCCGACGTGCCTGCCTCGGCCCGCCAGGAGGGGTGCCAGGACATCGCTACGCAGCT
180 A G T Y A H T V N R N W Y S D A D V P A S A R Q E G C Q D I A T Q L

1301 CATCTCCAACATGGACATTGATGTGATCCTGGGTGGAGGCCGAAAGTACATGTTTCGCATGGGAACCCCAGACCCTGAGTACCCAGATGACTACAGCCAA
213 I S N M D I D V I L G G G R K Y M F R M G T P D P E Y P D D Y S Q

1401 GGTGGGACCAGGCTGGACGGGAAGAATCTGGTGCAGGAATGGCTGGCGAAGCGCCAGGGTGCCCGGTATGTGTGGAACCGCACTGAGCTCATGCAGGCTT
247 G G T R L D G K N L V Q E W L A K R Q G A R Y V W N R T E L M Q A

1501 CCCTGGACCCGTCTGTGACCCATCTCATGGGTCTCTTTGAGCCTGGAGACATGAAATACGAGATCCACCGAGACTCCACACTGGACCCCTCCCTGATGGA
280 S L D P S V T H L M G L F E P G D M K Y E I H R D S T L D P S L M E

SacII (1643)
1601 GATGACAGAGGCTGCCCTGCGCCTGCTGAGCAGGAACCCCCGCGGCTTCTTCCTCTTCGTGGAGGGTGGTCGCATCGACCACGGTCATCACGAAAGCAGG
313 M T E A A L R L L S R N P R G F F L F V E G G R I D H G H H E S R

1701 GCTTACCGGGCACTGACTGAGACGATCATGTTCGACGACGCCATTGAGAGGGCGGGCCAGCTCACCAGCGAGGAGGACACGCTGAGCCTCGTCACTGCCG
347 A Y R A L T E T I M F D D A I E R A G Q L T S E E D T L S L V T A

1801 ACCACTCCCACGTCTTCTCCTTCGGAGGCTACCCCCTGCGAGGGAGCTCCATCTTCGGGCTGGCCCCTGGCAAGGCCCGGGACAGGAAGGCCTACACGGT
380 D H S H V F S F G G Y P L R G S S I F G L A P G K A R D R K A Y T V

1901 CCTCCTATACGGAAACGGTCCAGGCTATGTGCTCAAGGACGGCGCCCGGCCGGATGTTACCGAGAGCGAGAGCGGGAGCCCCGAGTATCGGCAGCAGTCA
413 L L Y G N G P G Y V L K D G A R P D V T E S E S G S P E Y R Q Q S

2001 GCAGTGCCCCTGGACGAAGAGACCCACGCAGGCGAGGACGTGGCGGTGTTCGCGCGCGGCCCGCAGGCGCACCTGGTTCACGGCGTGCAGGAGCAGACCT
447 A V P L D E E T H A G E D V A V F A R G P Q A H L V H G V Q E Q T

2101 TCATAGCGCACGTCATGGCCTTCGCCGCCTGCCTGGAGCCCTACACCGCCTGCGACCTGGCGCCCCCCGCCGGCACCACCGACGCCGCGCACCCGGGGCG
480 F I A H V M A F A A C L E P Y T A C D L A P P A G T T D A A H P G R

NheI (2227)
2201 GTCCCGGTCCAAGCGTCTGGATTGAAGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGAAAAAAATG
513 S R S K R L D •

2301 CTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCATTCATTTTATGTTTCAG

2401 GTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGGAATTAATTCTAAAATACAGCATAGCAAAACTTTAACCTC

2501 CAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTC
2601 TTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAACTAGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAG
2701 TAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCCA
2801 GTTTAGTAGTTGGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACA
125 • D Q E E A V

2901 AAGTGCACGCAGTTGCCGGCCGGGTCGCGCAGGGCGAACTCCCGCCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGT
117 F H V C N G A P D R L A F E R G W P Q E G I E T M A P G S A D R F N

3001 TCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGC
84 T S V V E S W E A Y L E D L G R V W V W A L T N D P V V Q D Q V A

SgrAI (3134)
3101 GCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCGGTCCAGAACTCGACCGCT
51 S I F L T V D 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

3201 CCGGCGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGATGGCTCCTCCTGTCAGGAGAGGAAAGAGAAGAAGGTTAGTACAAT
17 G A V D R A T L V P V A S T L K A M

3301 TGCTATAGTGAGTTGTATTATACTATGCAGATATACTATGCCAATGATTAATTGTCAAACTAGGGCTGCAGGTTAATTAAGAACATGTGAGCAAAAGGCC

3401 AGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAG

3501 GTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTG

3601 TCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGC

3701 ACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCAC

3801 TGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATC

3901 TGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGC

4001 AGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTT

4101 GGTCATGGCTAGTTAATTAACATTTAAATCA