Human COCH ORF mammalian expression plasmid, N-His tag

Catalog Number: HG11368-NH

General Information
Gene: coagulation factor C homolog, cochlín (Limulus polyphemus)
Official Symbol: COCH
Synonym: DFNA9, COCH5B2, COCH-5B2, COCH
Source: Human
cDNA Size: 1653bp
RefSeq: NM_004086.2

Description
Lot: Please refer to the label on the tube
Vector: pCMV3-SP-N-His
Shipping carrier: Each tube contains approximately 10 μg of lyophilized plasmid.

Storage:
The lyophilized plasmid can be stored at ambient temperature for three months.

Quality control:
The plasmid is confirmed by full-length sequencing with primers in the sequencing primer list.

Sequencing primer list:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCMV3-F</td>
<td>5’ CAGGTGCCACTCCAGGTCCAAG 3’</td>
</tr>
<tr>
<td>pcDNA3-R</td>
<td>5’ GCGACTAGAAGGCACAGTGG 3’</td>
</tr>
<tr>
<td>Forward T7</td>
<td>5’ TAATACGACTCATAGGG 3’</td>
</tr>
<tr>
<td>ReverseBGH</td>
<td>5’ TAGAAGGACAGTGG 3’</td>
</tr>
</tbody>
</table>

Or

pCMV3-F and pcDNA3-R are designed by Sino Biological Inc. Customers can order the primer pair from any oligonucleotide supplier.

Plasmid Resuspension protocol

1. Centrifuge at 5,000× g for 5 min.
2. Carefully open the tube and add 100 μl of sterile water to dissolve the DNA.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000× g.
5. Store the plasmid at -20 °C.

The plasmid is ready for:

- Restriction enzyme digestion
- PCR amplification
- E. coli transformation
- DNA sequencing

E. coli strains for transformation (recommended but not limited)

Most commercially available competent cells are appropriate for the plasmid, e.g. TOP10, DH5α and TOP10F’.
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Vector Information

All of the pCMV vectors are designed for high-level stable and transient expression in mammalian hosts. High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human enhanced cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells.
- Hygromycin resistance gene for selection of mammalian cell lines.
- A Kozak consensus sequence to enhance mammalian expression.

<table>
<thead>
<tr>
<th>Vector Name</th>
<th>pCMV3-SP-N-His</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector Size</td>
<td>6149bp</td>
</tr>
<tr>
<td>Vector Type</td>
<td>Mammalian Expression Vector</td>
</tr>
<tr>
<td>Expression Method</td>
<td>Constitutive, Stable / Transient</td>
</tr>
<tr>
<td>Promoter</td>
<td>CMV</td>
</tr>
<tr>
<td>Antibiotic Resistance</td>
<td>Kanamycin</td>
</tr>
<tr>
<td>Selection In Mammalian Cells</td>
<td>Hygromycin</td>
</tr>
<tr>
<td>Protein Tag</td>
<td>His</td>
</tr>
</tbody>
</table>
**Physical Map**

```
<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Kan(R)</td>
<td>CMV promoter</td>
</tr>
<tr>
<td>enhancer</td>
<td>T7 primer</td>
</tr>
<tr>
<td>enhancer</td>
<td>Kozak</td>
</tr>
<tr>
<td>Signal Peptide</td>
<td>N-His</td>
</tr>
<tr>
<td>linker</td>
<td>MCS</td>
</tr>
<tr>
<td>BGH reverse primer</td>
<td></td>
</tr>
<tr>
<td>Sv40 early promoter</td>
<td></td>
</tr>
<tr>
<td>Hygro(R)</td>
<td></td>
</tr>
</tbody>
</table>
```

**pCMV3-SP-N-His (suitable for secretary and membrane protein expression)**

**Description**

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<td>His</td>
</tr>
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<td>Sequencing Primer</td>
<td>Forward: T7(TAATACGACTCACTATAGGG) Reverse: BGH(TAGAAGGCACAGTCGAGG)</td>
</tr>
</tbody>
</table>

**Schematic of pCMV3-SP-N-His Multiple Cloning Sites**

```
1415
GGTGCACCTCCAGGTCAGCAGTTTTAACAATTTATATACGACTCATATAGGG
Kozak
GCCGCCACC

1475
AAG CTT
GGT ACC
ATGCCACGTGCTGCTGGGCTGGAGCTCTGCTGCT
Hind III
Kpn I

1535
CAG CAT CAC CAT CAT CAC CAT CAC CAT CAC
GGG GGA GGC GGT
AGC GCT

1583
GCT AGC
GGG TCC
GT TAC
AAT I
Hind III

1620
GCG TAC
TCT AGA
GGG GCC GCC
GAATT C GGG CCC
GTTT AAAC

1670
CGCTGTAGCATCAGCTGACTGTCCCTGTTA
GCCGCCACCATCTGTTTTGC
```

**Comments for pCMV3-SP-N-His:**

- CMV promoter: bases 250-837
- enhancer: bases 838-1445
- SV40 early promoter: bases 2390-2759
- Hygromycin ORF: bases 2777-3802
- pUC origin: bases 4445-5118
- Kanamycin ORF: bases 5192-6007

**pCMV3-SP-N-His is recommended for constructing the N-His tag secretary and membrane proteins expression vector which containing a naïve signal peptide. An universal signal peptide is used to instead the naïve signal peptide.**