Influenza A H5N1 (A/Duck/Hong Kong/p46/97) Hemagglutinin ORF mammalian expression plasmid, N-Flag tag

Catalog Number: VG40001-NF

General Information

Gene: H5N1 (A/Duck/Hong Kong/p46/97) HA
Official Symbol: HA
Synonym: HA1, Hemagglutinin
Source: H5N1
cDNA Size: 1725bp
Plasmid: pCMV3-Flag-H5N1-DH-97-HA

Description

Lot: Please refer to the label on the tube

Sequence Description:

A number of silent mutations were introduced into the DNA sequence in order to increase its protein expression level in mammalian cell system. The translated amino acid sequence is identical with AAF02306.1.

Restriction site: HindIII + XbaI (6kb + 1.82kb)
Vector: pCMV3-SP-N-FLAG

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’ CAGGTGTCCACTCCAGGTCAAG 3’</td>
</tr>
<tr>
<td>pcDNA3-R</td>
<td>5’ GGCAACTAGAAGGCACAGTGGG 3’</td>
</tr>
</tbody>
</table>

Or

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward T7</td>
<td>5’ TAATACGACTCATATAGGG 3’</td>
</tr>
<tr>
<td>Reverse BGH</td>
<td>5’ TAGAAGGCACAGTGGG 3’</td>
</tr>
</tbody>
</table>

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.

Physical Map of Plasmid:

Vector Name: pCMV3-SP-N-FLAG
Vector Size: 6143bp
Vector Type: Mammalian Expression Vector
Expression Method: Constitutive, Stable/Transient
Promoter: CMV
Antibiotic Resistance: Kanamycin
Selection in Mammalian Cells: Hygromycin
Protein Tag: FLAG
**pCMV3-SP-N-FLAG** (suitable for secretory and membrane protein expression)

### Physical Map

- **pCMV-Sp-N-Flag**
- CMV promoter
- Enhancer
- T7 primer
- Kozak
- Signal Peptide
- N-Flag
- Linker
- MCS
- BGH reverse primer
- Kan(R)
- Hygro(R)
- SV40 early promoter
- 6143 bp

### Description

- **Vector Name**: pCMV3-SP-N-FLAG
- **Vector Size**: 6143 bp
- **Vector Type**: Mammalian Expression Vector
- **Expression Method**: Constitutive, Stable / Transient
- **Promoter**: CMV
- **Antibiotic Resistance**: Kanamycin
- **Selection In Mammalian Cells**: Hygromycin
- **Protein Tag**: FLAG
- **Sequencing Primer**:
  - Forward: T7(TAATACGACTCACTATAGGG)
  - Reverse: BGH(TAGAAGGCACAGTCGAGG)

### Comments for pCMV3-SP-N-FLAG:

- CMV promoter: bases 250-837
- Enhancer: bases 838-1445
- SV40 early promoter: bases 2384-2753
- Hygromycin ORF: bases 2771-3793
- pUC origin: bases 4439-5112
- Kanamycin ORF: bases 5186-6001

### Schematic of pCMV3-SP-N-FLAG Multiple Cloning Sites

- **T7 primer binding site**
- **Kozak**
- **Signal Peptide**
- **Linker**
- **AatII**
- **Stop Codon**
- **Xba I**
- **Not I**
- **Apa I**

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