Influenza A H5N1 (Thailand/1(KAN-1)/2004) Hemagglutinin / HA (Codon Optimized) ORF mammalian expression plasmid, N-HA tag

Catalog Number: VG40065-NY

General Information

Gene: H5N1 (A/Thailand/1(KAN-1)/2004) Hemagglutinin

Official Symbol: HA

Synonym: Hemagglutinin, HA

Source: H5N1 cDNA

cDNA Size: 1707bp

Description

Lot: Please refer to the label on the tube

Vector: pCMV3-SP-N-HA

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>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCMV3-F</td>
<td>5’ CAGGTGTCCACTCCAGGTCAAAG 3’</td>
</tr>
<tr>
<td>pcDNA3-R</td>
<td>5’ GGCAACTAGAAGGCACAGTCGAGG 3’</td>
</tr>
</tbody>
</table>

Or

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward T7</td>
<td>5’ TAATACGACTCACTATAGGG 3’</td>
</tr>
<tr>
<td>ReverseBGH</td>
<td>5’ TAGAAGGCACAGTCGAGG 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−.
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.

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<th>Vector Name</th>
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<tr>
<td>Vector Size</td>
<td>6146bp</td>
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<tr>
<td>Vector Type</td>
<td>Mammalian Expression Vector</td>
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<tr>
<td>Expression Method</td>
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<td>Promoter</td>
<td>CMV</td>
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<td>Antibiotic Resistance</td>
<td>Kanamycin</td>
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<td>Selection In Mammalian Cells</td>
<td>Hygromycin</td>
</tr>
<tr>
<td>Protein Tag</td>
<td>HA</td>
</tr>
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</table>
**pCMV3-SP-N-HA** (suitable for secretary and membrane protein expression)

**Physical Map**

- CMV promoter
- Enhancer
- TT primer
- Kozak
- Signal Peptide
- N-HA
- Linker
- MCS
- BGH reverse primer
- SV40 early promoter
- Hygro (R)
- Kan (R)

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<td>Sequencing Primer</td>
<td>Forward: T7(TAATACGACTCACTATAGGG) Reverse: BGH(TAGAAGGCACAGTCGAGG)</td>
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**Schematic of pCMV3-SP-N-HA Multiple Cloning Sites**

- **1145**: GGTGTCACCTCCAGSTCCAAATTTAAACTTTAATACGACTCACTATAGGG GCCGCCACC
- **1175**: Hind III Kpn I
- **1535**: TAT CCT TAC GAC GTG CTT CAC TAC GCC GTG GGA GCC GGT AGC GCT
- **1588**: Nhe I BamHI Hind III Hpa II Afl II Age I Kex II Stop Codon
- **1620**: CTC GAG TCT AGA GCC GCC GCC GAATTC GGG CCC GTTT AAAC
- **1667**: CCCTCTAGAGCTGACCTGACGCTCTGATCCTTCTA GTTGCCAGCCATCTGTTTGGC

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

- CMV promoter: bases 250-837
- Enhancer: bases 838-1445
- SV40 early promoter: bases 2387-2756
- Hygromycin ORF: bases 2774-3799
- pUC origin: bases 4442-5115
- Kanamycin ORF: bases 5189-6004

**pCMV3-SP-N-HA** is recommended for constructing the N-HA 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.