**General Information**

**Gene:** H1N1 (A/New Caledonia/20/99) HA

**Official Symbol:** HA

**Synonym:** HA1, Hemagglutinin

**Source:** H1N1

**cDNA Size:** 1698bp

**Plasmid:** pCMV3-Myc-H1N1-NC-99-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 AAP34324.1.

**Restriction site:** KpnI + XbaI (6kb + 1.74kb)

**Vector:** pCMV3-SP-N-Myc

**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’ CAGGTGTCCACTCCAGGTAAG 3’</td>
</tr>
<tr>
<td>pcDNA3-R</td>
<td>5’ GGCAAATGGAAGGCACAGTCCAGG 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’ TAGAAGGCACAGTCAGG 3’</td>
</tr>
</tbody>
</table>

**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.

Physical Map of Plasmid:

[Diagram of plasmid with labeled elements: Kan(R), ori, CMV promoter, enhancer, T7 primer, KpnI, Signal Peptide, N-Myc linker, ORF, Hygro(R), SV40 early promoter, XbaI, BGH reverse primer]
**Physical Map**

- **CMV promoter**
- **T7 primer**
- **Kozek**
- **Signal Peptide**
- **M-Nyc**
- **linker**
- **MCS**
- **BGH reverse primer**

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

- 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

**Description**

<table>
<thead>
<tr>
<th>Vector Name</th>
<th>pCMV3-SP-N-Myc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vector Size</td>
<td>6149bp</td>
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<tr>
<td>Vector Type</td>
<td>Mammalian Expression Vector</td>
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<tr>
<td>Expression Method</td>
<td>Constitutive, Stable / Transient</td>
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<tr>
<td>Promoter</td>
<td>CMV</td>
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<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>Myc</td>
</tr>
<tr>
<td>Sequencing Primer</td>
<td>Forward:T7(TAATACGACTCACTATAGGG)</td>
</tr>
<tr>
<td></td>
<td>Reverse:BGH(TAGAAGGCCACAGTCGAGG)</td>
</tr>
</tbody>
</table>

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

pCMV3-SP-N-Myc is recommended for constructing the N-Myc 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.