Rat NGF ORF mammalian expression plasmid, N-Flag tag

Catalog Number: RG80426-NF

General Information
Gene: nerve growth factor (beta polypeptide)
Official Symbol: NGF
Synonym: Ngfb, Ngf
Source: Rat
cDNA Size: 726bp
RefSeq: XM_003749364.1
Plasmid: pCMV3-Flag-ratNGF

Description
Lot: Please refer to the label on the tube
Sequence Description:
Identical with the Gene Bank Ref. ID sequence.
Restriction site: KpnI + XbaI (6kb + 0.81kb)
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’ CAGGTGTCCACTCCCAGGTCCAAG 3’</td>
</tr>
<tr>
<td>pcDNA3-R</td>
<td>5’ GGCAACTAGAAGGCACTGAGG 3’</td>
</tr>
</tbody>
</table>

Or

<table>
<thead>
<tr>
<th>Primer</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Forward T7</td>
<td>5’ TAATACGACTCTATAGGG 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’.
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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

Physical Map of Plasmid:

- pCMV3-SP-Flag-ORF
- CMV promoter
- Kan(R)
- ori
- enhancer
- T7 primer
- KpnI
- Signal Peptide
- N-Flag
- linker
- ORF
- Hygro(R)
- SV40 early promoter
- XhoI
- BGH reverse primer
**Physical Map**

**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

**Description**

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

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