Rat EPO ORF mammalian expression plasmid, N-HA tag

Catalog Number: RG80055-NY

General Information

**Gene:** erythropoietin

**Official Symbol:** EPO

**Synonym:** Epo

**Source:** Rat

**cDNA Size:** 579bp

**RefSeq:** NM_017001.1

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:**

- pCMV3-F: 5’ CAGGTGTCCACTCCAGGTCCAGG 3’
- pcDNA3-R: 5’ GGCAACTAGAAGGCACAGTCGAGG 3’

Or

- Forward T7: 5’ TAATACGACTACTATAGGG 3’
- ReverseBGH: 5’ TAGAAGGCACAGTCGAGG 3’

**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>
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<th>Vector Name</th>
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<tbody>
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<td>6146bp</td>
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Rat EPO ORF mammalian expression plasmid, N-HA tag
**pCMV3-SP-N-HA** (suitable for secretary and membrane protein expression)

**Physical Map**

![Physical Map Diagram]

**Description**

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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**

```
1415  GGTGTCACCTCGAGGTCAAGTTAACTTTATACAGCTACCTATAGGG GCGGCCAG

1417  Kpn I   Hind III  T7 promoter binding site

1435  Human CD33 Signal Peptide

1500  Nhe I   BamHI   Hind III   Age I   Hpa II   Xba I   Xho I  

1525  Nhe I   BamHI   Hind III   Age I   Hpa II   Xho I  

1620  CTC GAG   TCT AGA   GCG GCC   GGC CCG   GAT ATC   ATC GAT   TAA A

1667  CGCCTGACATCGCTGACACTGCTTTCTA GTGCGCAGCAGATGTAGTTGCG
```

**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.