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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**General Information**

**Gene:** poliovirus receptor  
**Official Symbol:** PVR  
**Synonym:** PVS, mE4, HVED, Taa1, CD155, Tage4, necl-5, D7Ertd458e, 3830421F03Rik, Pvr  
**Source:** Mouse  
**cDNA Size:** 1227bp  
**RefSeq:** NM_027514.1  
**Plasmid:** pCMV3-mPVR-OFPSpark

**Description**

**Lot:** Please refer to the label on the tube  
**Sequence Description:**  
Identical with the Gene Bank Ref. ID sequence.  
**Restriction site:** KpnI (two restriction sites) + XbaI (6kb+1.29kb+0.63kb)  
**Vector:** pCMV3-C-OFPSpark  
**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 Type</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCMV3-F</td>
<td>5’ CAGGTGTCCACTCCACGTCAAG 3’</td>
</tr>
<tr>
<td>pcDNA3-R</td>
<td>5’ GGCAACTAGAAGGACAGTCGAGG 3’</td>
</tr>
<tr>
<td>Forward T7</td>
<td>5’ TAAACTCAGACTCATATAGGG 3’</td>
</tr>
<tr>
<td>Reverse BGH</td>
<td>5’ TAAAGGACAGTCGAGG 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.
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-C-OFPSpark
Vector Size: 6806bp
Vector Type: Mammalian Expression Vector
Expression Method: Constitutive, Stable / Transient
Promoter: CMV
Antibiotic Resistance: Kanamycin
Selection in Mammalian Cells: Hygromycin
Protein Tag: OFPSpark
Sequencing Primer: Forward: T7(TAATACGACTCACTATAGGG)
Reverse: BGH(TAGAAGGCAACGTCGAGG)