SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike-Pseudotyped Lentiviral Kit V2

Catalog No. NR-53816

For research use only. Not for use in humans.

Contributor:
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Manufacturer:
BEI Resources

Product Description:
NR-53816 is intended for producing pseudotyped particles/pseudovirions and is not for soluble protein expression.

The severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), Wuhan-Hu-1 (GenBank: NC_045512) spike-pseudotyped lentiviral kit version 2 (NR-53816) is designed to generate pseudotyped lentiviral particles with the spike (S) glycoprotein gene, as well as luciferase (Luc2) and green fluorescent protein (GFP). Version 2 replaces the full-length S glycoprotein with a C-terminally truncated spike, which increases titers of viral particles pseudotyped with SARS-CoV-2 spike glycoprotein. Protocols for the use of these items are published, and updates are available at protocols.io.

NR-53816 consists of the five plasmids listed in Table 1. Descriptions of each component are included below.

Table 1: SARS-CoV-2 Lentiviral Kit

<table>
<thead>
<tr>
<th>Plasmid Type</th>
<th>Insert</th>
<th>BEI Resources Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral Entry Protein</td>
<td>S Glycoprotein ΔCter</td>
<td>NR-53742</td>
</tr>
<tr>
<td>Lentiviral Backbone</td>
<td>Luc2; ZsGreen</td>
<td>NR-52516</td>
</tr>
<tr>
<td>Helper Plasmid</td>
<td>Gag; pol</td>
<td>NR-52517</td>
</tr>
<tr>
<td>Helper Plasmid</td>
<td>Tat1b</td>
<td>NR-52518</td>
</tr>
<tr>
<td>Helper Plasmid</td>
<td>Rev1b</td>
<td>NR-52519</td>
</tr>
</tbody>
</table>

NR-52516 was designed by fusing the synthetic firefly luciferase (Luc2) gene to the encephalomyocarditis internal ribosomal entry site (IRES) and synthetic Zoanthus sp. green fluorescent protein (ZsGreen1) gene, allowing simultaneous expression of Luc2 and the ZsGreen1 gene, which were subcloned into the pHAGE lentiviral backbone vector under the CMV promoter. The Luc2 gene has been codon optimized for mammalian expression and has had cryptic transcription factor binding sites removed. The ZsGreen1 gene has been codon optimized for mammalian expression and engineered for brighter fluorescence. In addition, the pHAGE vector includes the Woodchuck hepatitis virus post-transcriptional regulatory element to enhance levels of transcription and gene expression. The resulting size of the plasmid is approximately 9370 base pairs.

NR-52517 and NR-52518 were designed by codon optimizing the genes gag and pol and the gene tat1b, respectively, from the human immunodeficiency virus (HIV) and subcloning them into the pHDM vector under the CMV promoter. The resulting plasmid sizes are approximately 8910 base pairs and 4830 base pairs, respectively.

NR-52519 was designed by codon optimizing the rev1b gene from HIV and subcloning into the pRC-CMV vector under the CMV promoter. NR-52519 contains a neomycin (G418) selectable marker for mammalian expression. The resulting size of the plasmid is approximately 5900 base pairs.

NR-53742, NR-52516, NR-52517, NR-52518 and NR-52519 contain the beta-lactamase gene, bla, to provide transformatant selection through ampicillin resistance in Escherichia coli (E. coli).

The complete plasmid sequences and maps are provided on the BEI Resources webpage. The plasmids were produced in E. coli and extracted.

Material Provided:
Each kit contains one vial of each plasmid DNA in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0. The DNA concentrations and volumes provided are shown on the Certificate of Analysis. The vials should be centrifuged prior to opening. Note: The contents of each vial should be used to replicate the plasmid in E. coli prior to mammalian expression.

Packaging/Storage:
NR-53816 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -20°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

Citation:
Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike-Pseudotyped Lentiviral Kit V2, NR-53816.”
Biosafety Level: 1

Disclaimers:
You are authorized to use this product for research use only. It is not intended for human use.

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NR-53816 is claimed in U.S. Patent number 8,008,006 and European Patent number 1341808 and the continuations, continuations-in-part, re-issues and foreign counterparts thereof.

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References: