Product Information Sheet for NR-53762

SARS-Related Coronavirus 2, USA-WA1/2020 Recombinant Infectious Molecular Clone Plasmid Kit

Catalog No. NR-53762

For research use only. Not for use in humans.

Manufacturer:
BEI Resources

Contributor:
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Product Description:

Note: The vial labels for NR-53752 to NR-53758 indicate these products are a molecular clone in vector pU57. The correct vector is pUC57 and each plasmid produces a viral fragment that must be combined with additional fragments to produce the molecular clone. The NR-53755 label also lists this clone as wildtype (WT); however, NR-53755 is not WT and includes a T15102A silent mutation.

The vectors for the recombinant infectious molecular clone from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), USA-WA1/2020 (GenBank: MT461669) was designed by RT-PCR amplification of SARS-CoV-2 virus (GenBank: MT020880) with restriction sites and four-nucleotide cohesive ends at the 5' and 3' insert termini and subcloned into the pUC57 expression vector,1,2

NR-53762 can be used to assemble recombinant infectious SARS-CoV-2, USA-WA/2020 and consists of the eight plasmids listed in Table 1. Descriptions of each component are included below.

Table 1: SARS-CoV-2 Molecular Clone Plasmid Kit

<table>
<thead>
<tr>
<th>Plasmid Type</th>
<th>Insert</th>
<th>BEI Resources Catalog Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus fragment</td>
<td>cDNA fragment A</td>
<td>NR-53752</td>
</tr>
<tr>
<td>Virus fragment</td>
<td>cDNA fragment B</td>
<td>NR-53753</td>
</tr>
<tr>
<td>Virus fragment</td>
<td>cDNA fragment C</td>
<td>NR-53754</td>
</tr>
<tr>
<td>Virus fragment</td>
<td>cDNA fragment D</td>
<td>NR-53755</td>
</tr>
<tr>
<td>Virus fragment</td>
<td>cDNA fragment E</td>
<td>NR-53756</td>
</tr>
<tr>
<td>Virus fragment</td>
<td>cDNA fragment F</td>
<td>NR-53757</td>
</tr>
<tr>
<td>Virus fragment</td>
<td>cDNA fragment G</td>
<td>NR-53758</td>
</tr>
<tr>
<td>Helper Plasmid</td>
<td>sgRNA-N</td>
<td>NR-53761</td>
</tr>
</tbody>
</table>

NR-53752 to NR-53755 and NR-53758 were designed with BsaI restriction sites for plasmid excision. The cDNA fragment A contains nucleotides 1 to 5415 as well as an upstream T7 promoter sequence, fragment B contains nucleotides 5406 to 10456, fragment C contains nucleotides 10450 to 14498, fragment D contains nucleotides 14493 to 17848 as well as a T15102A silent mutation and fragment G contains nucleotides 25412 to 29895 as well as a 25 nucleotide poly-A tail downstream.

NR-53756 and NR-53757 were designed with BsmBI restriction sites for plasmid excision. The cDNA fragment E contains nucleotides 17842 to 21497 and fragment F contains nucleotides 21492 to 25417.

NR-53761 is an sgRNA-N plasmid designed to enhance the efficiency of recovering SARS-CoV-2 virus in the cell culture. This plasmid includes a 75 base pair leader sequence, SARS-CoV-2 virus nucleocapsid (N) gene, 3' untranslated region (UTR) and a 25-nucleotide poly-A tail under control of a T7 promoter.

All plasmids contain the beta-lactamase gene, bla, to provide transformant 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-53762 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, USA-WA1/2020 Recombinant Infectious Molecular Clone Plasmid Kit, NR-53762.”

Biosafety Level: 1


Note: Infectious viral particles produced by use of this kit are a BSL3 organism. Virus production should be performed with appropriate biosafety controls.
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References:
1. Baric, R. S., Personal Communication.

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