

**Bacterial Artificial Chromosome Plasmid pSynkRSV-I19F Containing Antigenomic cDNA from Respiratory Syncytial Virus (RSV) A2-Line19F**

**Catalog No. NR-36460**

**Product Description:** Antigenomic cDNA of RSV A2-line19F was synthesized in three segments which were cloned sequentially into pKBS2 (a bacterial artificial chromosome, BAC, vector) to generate the pSynkRSV-I19F plasmid. The gene for the far-red fluorescent protein monomeric Katushka 2 was included in the RSV antigenomic DNA enable detection of infection through fluorescence.

**Lot<sup>1</sup>: 61047085**

**Manufacturing Date: 14MAR2013**

TEST	SPECIFICATIONS	RESULTS
<b>Appearance</b>	Report results	Clear and colorless
<b>Confirmation of Plasmid Integrity by Restriction Digestion<sup>2</sup></b> NR-36460 ( <i>EcoRI</i> digested)	123, 527, 762, 813, 1446, 2651, 7216, 9082 base pair bands	123, 527, 762, 813, 1446, 2651, 7216, 9082 base pair bands
<b>Functional Activity<sup>3</sup></b> RSV replication in transfected BHK-21 clone BSR T7/5 cells (3 days post-transfection)	Red fluorescent syncytia observed	Red fluorescent syncytia observed (Figure 1)
<b>PicoGreen<sup>®</sup> Measurement</b> DNA content DNA concentration	0.5 µg 0.1 µg per µL	0.5 µg 0.1 µg per µL
<b>OD<sub>260</sub>/OD<sub>280</sub> Ratio</b>	1.7 to 2.0	1.85
<b>Contamination with <i>E. coli</i> Genomic DNA</b>	Report results	~ 50% <sup>4</sup>
<b>Endotoxin</b>	≤ 50 EU/mL	≤ 0.9 EU/mL
<b>Bioburden [colony forming units (cfu)]</b>	≤ 1 cfu per 5 µL	0 cfu per 5 µL

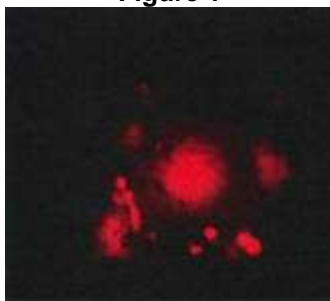
<sup>1</sup>Produced in *E. coli*, strain SW102 and extracted using NucleoBond<sup>®</sup> BAC 100 (Macherey-Nagel) plasmid DNA purification kit (cat # 740579).

<sup>2</sup>Undigested NR-36460 is 22620 base pairs.

<sup>3</sup>Recombinant RSV was produced by co-transfection of BHK-21 clone BSR T7/5 cells with NR-36460 and four helper plasmids containing genes for A2 large polymerase (NR-36461), nucleoprotein (NR-36462), phosphoprotein (NR-36463) and matrix 2-1 protein (NR-36464).

<sup>4</sup>BAC Maxi preparations have a higher level of contaminating genomic DNA than standard, high copy plasmid preparations because BACs are single copy in *E. coli*. The manufacturer predicted that the level of genomic DNA contamination would be less than 5%. The pSynkRSV-I19F plasmid produced functional, infectious RSV.

**Figure 1**



## Certificate of Analysis for NR-36460

**Date:** 24 SEP 2013

**Signature:**

*Dorothy C. Young*

**Title:** Technical Manager, BEI Authentication or designee

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