

Certificate of Analysis for NR-36460

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.

Lot¹: 61047085

Manufacturing Date: 14MAR2013

TEST	SPECIFICATIONS	RESULTS
Appearance	Report results	Clear and colorless
Confirmation of Plasmid Integrity by Restriction Digestion ² 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
Functional Activity ³ 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)
PicoGreen [®] Measurement DNA content DNA concentration	0.5 µg 0.1 µg per µL	0.5 µg 0.1 µg per µL
OD ₂₆₀ /OD ₂₈₀ Ratio	1.7 to 2.0	1.85
Contamination with <i>E. coli</i> Genomic DNA	Report results	~ 50% ⁴
Endotoxin	≤ 50 EU/mL	≤ 0.9 EU/mL
Bioburden [colony forming units (cfu)]	≤ 1 cfu per 5 µL	0 cfu per 5 µL

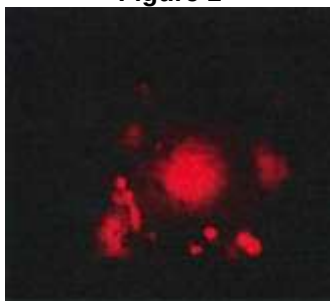
¹Produced in *E. coli*, strain SW102 and extracted using NucleoBond[®] BAC 100 (Macherey-Nagel) plasmid DNA purification kit (cat # 740579).

²Undigested NR-36460 is 22620 base pairs.

³Recombinant 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).

⁴BAC 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:** **Title:** Technical Manager, BEI Authentication or designee

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