Product # R145

Respiratory Syncytial Virus with Firefly Luciferase (RSV-Luc5)

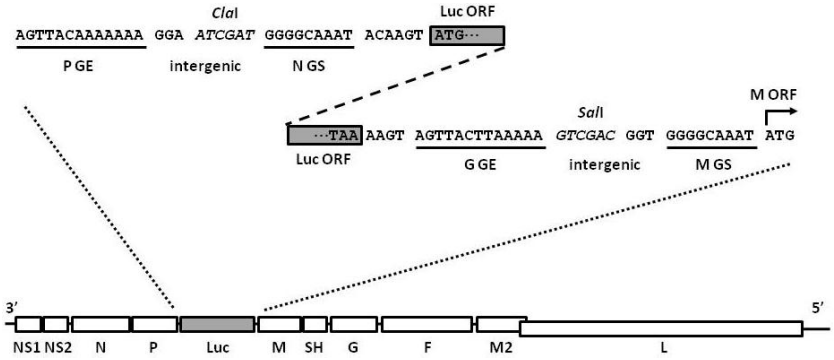
Introduction

As an addition to the second generation recombinant RSVs including RSV-GFP5 (Product #R125), RSV-DsRed5 (#R135), and RSV-FarRed5 (#R155), RSV-Luc5 was created to express Firefly luciferase (Luc) (Promega), the most widely used bioluminescent reporter. The position between the P and M genes was chosen for Luc insertion because it places the foreign gene well downstream of the NS1 and NS2 genes, avoiding direct effects on the expression of NS1 and NS2. To construct a transcriptional cassette for the reporter gene, the G gene end signal was chosen because it is among the most efficient of these signals and would have the minimum impact on the viral transcriptional program; the gene start signals are more highly conserved, and that of N is typical and was used. RSV-Luc5 is well suited for studies that demand precise quantitation.

Description

Wild-type parent based on RSV strain A2 were modified to express firefly luciferase (Promega) from a gene cassette placed between the P and M genes. The open reading frame (ORF) for firefly luciferase gene was PCR amplified to add a ClaI site and an RSV gene start (GS) signal (from the N gene) upstream of the ORF and an RSV gene end (GE) signal (from the G gene) and SalI site downstream of the ORF. The ClaI-SalI fragment was further cloned through multiple steps between the P and M genes in the full-length RSV cDNA plasmid. Recombinant RSV-Luc5 was rescued by transfecting T7 polymerase-expressing BSR T7/5 cells with the full-length antigenome plasmid and plasmids expressing N, P, L, and M2-1 genes. The recovered RSV-Luc5 was further propagated in HEp-2 cells, the expression of firefly luciferase by infected cells was confirmed by bioluminiscence assay, and the sequence of the virus was confirmed in its entirety. RSV-Luc5 was found to replicate as efficiently as its biological parental strain in cell cultures.

REFERENCE: *unpublished.*



Specification

Parental Strain:	Strain A2
Construction:	Luc gene was inserted between P and M genes as the 5th gene.
Passage History:	The isolate was propagated in HEp-2 cells (ATCC CCL-23).
Infectivity:	Titer > 6.5 $\log_{10}$ $TCID_{50}$ per mL.
Volume/Storage:	2 x 1.2 mL per cryovial. Store at −80°C.
Quality Testing:	No bacteria, fungus, or mycoplasma detected. Endotoxin < 10 EU/mL.
Availability:	Bulk quantity and custom orders are available. Contact info@viratree.com.