Human Respiratory Syncytial Virus with Green Fluorescent Protein (RSV-GFP1)

Introduction

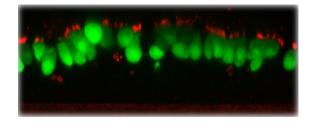
Human respiratory syncytial virus (RSV) is an enveloped, nonsegmented, negative-sense, single-stranded RNA virus belonging to the Pneumovirus genus of the subfamily Pneumovirinae, the family Paramyxoviridae. RSV is the most common virus responsible for acute and severe lower airway disease in infants and young children worldwide. Despite the enormous burden of RSV disease, there is no efficacious vaccine or antiviral drug therapy yet available. The RSV genome (15.2 kb) contains 10 mRNAs encoding 11 proteins. The nucleocapsid (N) protein binds the negative-strand RNA genome and associates with the phosphoprotein (P), the large (L) polymerase protein, and the M2-1 protein to form the nucleocapsid. The matrix (M) protein is present between the nucleocapsid and the outer envelope and plays a structural role in virion assembly and budding. There are three envelope glycoproteins: the attachment glycoprotein (G), the fusion (F) protein, and the small hydrophobic (SH) protein. The genome also encodes two nonstructural proteins (NS1, NS2) which suppress the interferon response and M2-2 protein (the second product of the M2 gene) which governs the transition from transcription to replication of genomic RNA.

Description

For the construction of RSV-GFP1, GFP (Green Lantern Protein, Life Technologies) was engineered to be flanked by RSV gene start and gene end sequences and was inserted as the first, promoter-proximal gene in a full-length cDNA of the wild-type RSV strain A2 antigenomic RNA(1). RSV-GFP1 was rescued by cotransfecting HEp-2 cells with the antigenomic plasmid and N, P, M2-1, and L support plasmids and infecting them with a modified vaccinia virus, MVA-T7, expressing T7 RNA polymerase. Rescued virus was amplified in HEp-2 cells, aliquoted and stored at -80°C. In cell cultures, RSV-GFP1 was found to replicate to near-parental titers and to produce syncytia at a rate similar to that of the parental virus. Using this virus, the main target cell type for RSV was found to be the ciliated epithelial cells in human airways (Fig.)(2).

REFERENCES

- Hallak LK, Spillmann D, Collins PL, Peeples ME. J Virol. 2000 Nov; 74(22):10508-13. PMID: 11044095
- Zhang L, Peeples ME, Boucher RC, Collins PL, Pickles RJ. J Virol. 2002 Jun; 76(11):5654-66. PMID: 11991994



Specification

Parental Strain: A2 strain

Construction: GFP gene was inserted in front of NS1 gene (as the first gene).

Passage History: The isolate was plaque purified and propagated in HEp-2 cells.

Infectivity: Titer > 6.0 \log_{10} TCID₅₀ per mL. Infectious in humans.

Volume/Storage: $2 \times 1.2 \text{ mL per cryovial}$. 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.