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

Catalog No. NR-36460

For research use only. Not for human use.

Contributor:
BEI Resources

Manufacturer:
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Product Description:
NR-36460 is a component of a bacterial artificial chromosome (BAC)-based RSV rescue system that allows RSV infection to be monitored by fluorescence and is an important tool in RSV vaccine research and mutagenesis studies. Please refer to Appendix I for the manufacturer's RSV rescue protocol.

Antigenomic cDNA of RSV A2-line19F was synthesized in three segments which were cloned sequentially into pKBS2 (BAC vector) to generate the pSynkRSV-I19F plasmid. The gene for the far-red fluorescent protein monomeric Katushka 2 (mKate2) was included in the RSV antigenomic DNA to enable detection of infection through fluorescence.[1,2] The plasmid was produced in *Escherichia coli*, strain SW102 cells[3] and extracted using a NucleoBond® BAC 100 (Macherey-Nagel) plasmid DNA purification kit.[2]

Material Provided:
Each vial contains 0.5 µg of plasmid DNA in RNase/DNase-free 10 mM Tris-HCl buffer (pH 8.5). The concentration is shown on the Certificate of Analysis. The vial should be centrifuged prior to opening.

Note: The protocol provided (see Appendix I) requires 0.8 µg of pSynkRSV-I19F; thus 2 vials of NR-36460 are required per transfection.

Packaging/Storage:
NR-36460 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -80°C or colder immediately upon arrival. Freeze-thaw cycles should be minimized.

Functional Activity:
Recombinant RSV was produced by co-transfection of BHK-21 clone BSR T7/5 cells[4] with NR-36460 and four helper plasmids containing sequence-optimized genes for RSV strain A2 large polymerase (NR-36461), nucleoprotein (NR-36462), phosphoprotein (NR-36463) and matrix 2-1 protein (NR-36464). RSV rescue and infection was detected by red fluorescent syncytia.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Bacterial Artificial Chromosome Plasmid pSynkRSV-I19F Containing Antigenomic cDNA from Respiratory Syncytial Virus (RSV) A2-Line19F, NR-36460."

Biosafety Level: 1
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:
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References:
1. Hotard, A. L., et al. "A Stabilized Respiratory Syncytial

Virus Reverse Genetics System Amendable to Recombination-Mediated Mutagenesis." Virology 434 (2012): 129-136. PubMed: 23062737.
2. M. L. Moore, Personnel Communication.
3. Warming, S., et al. "Simple and Highly Efficient BAC Recombineering using *galK* Selection." Nucleic Acids Res. 33 (2005): e36. PubMed: 15731329.
4. Buchholz, U. J., S. Finke and K. -K. Conzelmann. "Generation of Bovine Respiratory Syncytial Virus (BRSV) from cDNA: BRSV NS2 Is Not Essential for Virus Replication in Tissue Culture, and Human RSV Leader Region Acts as a Functional BRSV Genome Promoter." J. Virol. 73 (1999): 251-259. PubMed: 9847328.

Support Provided by NIAID

Appendix I

Transfection Procedure for Virus Recovery of Recombinant Respiratory Syncytial Virus

Materials (Suggested suppliers and catalog numbers are indicated):

BHK-21 clone BSR T7/5 cell cultures or alternative cells [BHK21 cells (ATCC® CCL10™) transfected with phage T7 polymerase from Modified Vaccinia Ankara (MVA)] Note: This protocol is optimized for use with BHK-21 clone BSR T7/5 cells. Use of alternative cells may result in decreased recovery of RSV.
Opti-MEM (serum-free) (Gibco/Life Technologies catalog #11058-021)
GMEM [Glasgow's MEM (Gibco/Life Technologies catalog #11710-035)] + 3% FBS
MEM non-essential amino acids (NEAA) 100X solution (Gibco/Life Technologies catalog #11140-050)
G418 sulfate, 50 mg/mL solution (500X) (Agilent Technologies Genomics catalog # 200049)
Trypsin-EDTA (0.25%) (Gibco/Life Technologies catalog #25200-072)
Antibiotic-Antimycotic solution, penicillin/streptomycin/amphotericin (100X) (Corning cellgro® catalog #30-004-Cl) or equivalent
Plasmid with RSV antigenome (NR-36460) each vial contains 0.5 µg in 5 µL total volume (Note: This protocol requires 0.8 µg of pSynkRSV-I19F; thus 2 vials of NR-36460 are required per transfection.)
Helper Plasmids – (all codon optimized) each vial contains 0.5 µg in 5 µL total volume:
pA2-Lopt, L protein (NR-36461)
pA2-Nopt, N protein (NR-36462)
pA2-Popt, P protein (NR-36463)
pA2-M2-1opt, M2-1 protein (NR-36464)
Lipofectamine 2000 transfection reagent (Gibco/Life Technologies catalog #11668-019)
Phosphate buffered saline pH 7.2 (Gibco/Life Technologies catalog #20012027)
6-well tissue culture plates
25 cm^2 tissue culture flasks
Shaker/rocker plate
Tissue culture humidified incubator with 3% to 5% CO_2
Assorted sterile pipettes and tips

Procedure:

Note: This protocol assumes the user is familiar with cell culture techniques and transfection procedures.

1. Initial cell culture:
   a. For routine sub-passage of BSR T7/5 cells, prepare new 25 cm^2 cultures at a ratio of one donor culture to three new cultures, based on surface area of the culture flasks (1:3 passage ratio). Use GMEM with 3% FBS + 1X NEAA + 1X antibiotics as growth medium, 5 mL per flask. When maintaining donor cultures, add 1X G418 to the growth medium every other passage.
   b. For transfections, sub-pass BSR T7/5 cells from "donor" cultures into 6 well plates so they will be 100% confluent at time of transfection. Use one 25 cm^2 culture to prepare one 6 well plate (1:2.5 passage ratio).

2. Prepare 6 well plates for transfection from 25 cm^2 donor cultures. Determine how many plates will be required and use the corresponding number of flasks. Aspirate the growth medium from the flasks, and then add 0.25 mL of warm trypsin-EDTA per 25 cm^2 flask. Rock flasks to distribute the trypsin-EDTA and incubate at 37°C for 5 to10 minutes. When cells start to dislodge from the flask, add 12 mL of GMEM with 3% FBS to each flask and use a pipet to suspend the cells in this growth medium. Add 2 mL of the cell suspension to each well in the 6 well plates. Incubate the plates at 37°C in the tissue culture incubator until the cell sheets are confluent and ready for transfection.

3. Prepare the reagents for the transfection procedure. Transfection will be done using Lipofectamine 2000 as the transfection reagent. Additionally, it is important to include control transfections (Lipofectamine only/wild type virus for mutants etc.)
   a. Use a 3:1 ratio of Lipofectamine (µL) to plasmid/helper plasmid (µg). Dilute each component with Opti-MEM to make 100 µL of each. After dilution, allow each dilution to sit at room temperature for 5 minutes.
   b. Use the following amounts of each component per transfection:
      i. RSV antigenome (NR-36460) 0.8 µg (8 µL of 0.1 µg/µL) + 92 µL Opti-MEM (2 vials of NR-36460 are required per transfection.)
      ii. pA2-Lopt, L protein (NR-36461) 0.2 µg (2 µL of 0.1 µg/µL) + 98 µL Opti-MEM
      iii. pA2-Nopt, N protein (NR-36462) 0.4 µg (4 µL of 0.1 µg/µL) + 96 µL Opti-MEM
      iv. pA2-Popt, P protein (NR-36463) 0.4 µg (4 µL of 0.1 µg/µL) + 96 µL Opti-MEM
      v. pA2-M2-1opt, M2-1 protein (NR-36464) 0.4 µg (4 µL of 0.1 µg/µL) + 96 µL Opti-MEM

vi. Lipofectamine 2000 6.6 µL + 93.4 µL Opti-MEM

Note: For multiple transfections increase the above quantities proportionally.

c. After allowing the diluted components to sit at room temperature for 5 minutes, combine all six components in one vial, mix gently and incubate the transfection mixture at room temperature for 20 minutes.
d. Transfection mixtures should be 600 µL total (Opti-MEM, Lipofectin, and DNA)
e. Aspirate the media from the BSR T7/5 cell culture plate, wash cells twice with 1 mL warm Opti-MEM for each wash, and aspirate the final wash.
f. Add 600 µL transfection mixture to each well and incubate the plate 2 hours at room temperature on a shaker/rocker plate set at low speed.
g. After 2 hours, add an additional 600 µL warm Opti-MEM per well and place plate in a 37°C tissue culture incubator overnight (8-12 hours).

4. After incubation, aspirate and discard the transfection mixture from the wells, wash each well once with 1 mL warm sterile PBS, aspirate the PBS and replace with 2 mL of warm GMEM with 3% FBS per well. Continue incubating at 37°C in the tissue culture incubator overnight.

5. Day 2 post transfection, sub-pass the cells into 25 cm^2 flasks using the trypsin-EDTA procedure described above. Pass at a 1:3 surface area ratio unless cell morphology appears weak, in which case the ratio should be decreased accordingly up to an even 1:1 ratio. (Note: surface area of each well in the 6 well plate is 10 cm^2). Cells should remain in GMEM with 3% FBS throughout the rest of recovery.

6. Monitor flasks for cytopathic effect (CPE) and sub-pass at 1:3 ratio into new 25 cm^2 flasks as needed (approximately every 48 hours). CPE shows first as mini-syncytia and then grows into rounded up clumps of cells.

7. When CPE is evident throughout the flask, scrape the cells into the growth media and aliquot into cryovials. Freeze at -80°C or colder.