

Nov 11, 2025

Version 2

Production of bunyavirus pseudotyped virus V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.5jyl88nkdI2w/v2>

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Protocol Citation: Giada Mattiuzzo, Emma Bentley, Federica Marchesin 2025. Production of bunyavirus pseudotyped virus. protocols.io <https://dx.doi.org/10.17504/protocols.io.5jyl88nkdI2w/v2> Version created by **Giada Mattiuzzo**

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Protocol status: Working

We use this protocol and it's working

Created: November 11, 2025



Last Modified: November 11, 2025

Protocol Integer ID: 232084

Keywords: virus several bunyavirales family, production of bunyavirus, prototype viruses for the family phenuiviridae, bunyavirus, rift valley fever virus, congo haemorrhagic fever virus, viral isolate, authentic infectious virus, alternative to authentic infectious virus, pseudotyped virus, haemorrhagic fever virus, viral glycoprotein, family phenuiviridae, class bunyaviricete, rapid provision of vaccine, early steps in virus entry, virus entry, prototype virus, virus, vaccine, pandemic preparedness, family nairoviridae, antibodies in serological assay, rvfv, neutralising antibody, serological assay

Abstract

Several *Bunyavirales* families have been listed as being of high pandemic or epidemic risk by the WHO R&D Blueprint. To support pandemic preparedness and the 100 Days Mission, along with rapid provision of vaccines and therapeutics, the development of tools to assess the immune response is required. Pseudotyped viruses (PV) have been shown to be a suitable alternative to authentic infectious virus to measure virus neutralising activity, a key component of the immune response. They alleviate the need to acquire and amplify viral isolates and do not require high containment facilities. Generating PV of some families within the class *Bunyaviricetes* is challenging because of a lack of co-localisation of viral glycoproteins at the vector budding site. This protocol describes a versatile plug-and-play system which has been successfully used for the pseudotyping of two prototype viruses for the family *Phenuiviridae*, Rift Valley fever virus (RVFV) and for the family *Nairoviridae*, Crimean-Congo haemorrhagic fever virus (CCHFV). The system was first described by Dr Michael A. Whitt in 2010 (MA Whitt, J Virol Methods 2010 Nov; 169(2): 365-374). The following protocol describes the generation of rVSV*Luc expressing a bunyavirus (e.g. RVFV, CCHFV) glycoprotein. The pseudotyped rVSV*Luc virus can be used to test early steps in virus entry or for the quantification of neutralising antibodies in serological assays.



Materials

Cell Lines

- HEK-293T/17 (ATCC, CRL-11268)

Culture Media

- DMEM (1X) + GlutaMAX (ThermoFisher: # 61965-026) or equivalent
- 10% v/v Foetal Calf Serum (Pan Biotech GmbH, P30-3306 Heat inactivated, South American origin) or equivalent
- 1% v/v Penicillin-Streptomycin (Sigma-Aldrich: # P0781) or equivalent

Plasmids/Recombinant Virus

- Expression plasmid containing virus GnGc gene
- Empty expression backbone plasmid (if required for Δ Env)
- G* Δ G-luciferase(luc) rVSV (Kerafast: # EH1020-PM) or similar

Reagents

- 0.25% Trypsin-EDTA Solution (Sigma-Aldrich: # T4049) or equivalent
- Opti-MEM I (1X) (ThermoFisher: # 31985-047) or equivalent
- DMEM (1X) + GlutaMAX (ThermoFisher: # 61965-026) or equivalent
- Phosphate Buffer Solution (ThermoFisher: # 10010023) or equivalent
- 1 mg/mL Polyethylenimine (PEI) (Sigma-Aldrich: # 408727) or equivalent

Consumables/Equipment

- 10 cm TC-treated culture dish (Corning: # 430167) or equivalent
- 0.45 μ m filter unit (Whatman: # 10462100) or equivalent
- 10 mL sterile syringe (BD Plastipak: # 302188) or equivalent
- Nunc MicroWell TC-treated 96-well microplate (ThermoFisher: #168055) or equivalent
- Polypropylene sterile conical tubes, 15 mL (Sarstedt: # 62.554.502) or equivalent
- 1.5 mL sterile micro-tubes (Sarstedt: # 72.692.005) or equivalent
- Incubator at 37°C, 5% CO₂

Safety warnings

⚠ Follow local H&S regulations. This is a GM activity

Day 1: Seed Cells in Preparation for Transfection

30m

- 1 Seed two (to include a negative control) 10-cm culture dishes with 4×10^6 HEK-293T/17 cells in 10 mL culture media, to reach 60-80% confluence the next day. More plates can be seeded, as desired, however each experiment requires a single negative control plate
- 2 Incubate overnight at 37°C, 5% CO₂
- 3 If not ready, prepare the PEI as follows: tare a balance with an empty 50 mL tube; within an MSC pipette approximately 1-2 mL of stock PEI into the tube; weigh the tube and add 10 mL of water for each gram of PEI; vortex for 5-10 minutes until the solution is homogenous; this is the stock at 100 mg/mL, which can be stored at 4°C for at least 2 years. To prepare the working stock dilute 1:100 in water. Mix using a vortex. The working stock is stable at 4°C for 2 years

Day 2 PM: Cell Transfection with Glycoprotein Expression Plasmid

1h

- 4 Pre-warm to ambient temperature culture media for HEK-293T/17 cells, Opti-MEM and 1 mg/mL PEI
- 5 Prepare and label two sterile 1.5 mL micro-tubes containing:
 - A. 200 uL Opti-MEM + 9 ug glycoprotein plasmid
 - B. 200 uL Opti-MEM + 9 ug empty plasmid (Δ Env negative control)
- 6 Briefly vortex to mix and pulse centrifuge
- 7 Prepare further two sterile 1.5 mL micro-tubes each containing 200 uL Opti-MEM and then add 60 uL of 1 mg/mL PEI transfection reagent directly into the centre, briefly vortex to mix
- 8 Transfer the Opti-MEM + PEI mix to the tubes containing Opti-MEM + plasmid DNA
- 9 Gently flick the tube to mix 3-4 times and incubate at ambient temperature for 20 minutes
- 10 During the incubation, gently replace the culture media on HEK-293T/17 cells without disrupting the cell monolayer with 8 mL of fresh media



- 11 Following incubation, add the Opti-MEM + PEI + DNA mix to the cells dropwise while gently agitating plate to ensure even dispersal
- 12 Incubate overnight at 37°C, 5% CO₂

Day 3 AM: Infection with G*ΔG-luc rVSV

3h

- 13 Pre-warm to ambient temperature, serum free DMEM, complete culture media for HEK-293T/17 cells and PBS (1X)
- 14 Retrieve a single-use aliquot of G*ΔG-luc rVSV from -80°C storage and let it thaw at ambient temperature
- 15 Prepare a 15 mL tube containing 5 mL serum free DMEM for each dish of transfected cells, including the negative control
- 16 Add a volume of G*ΔG-luc rVSV required to achieve an MOI of 0.1. Cell numbers at this stage should be based on a single cell doubling event since seeding (approximately 8×10^6 cells per 10 cm dish). *E.g. use 4 μL/dish of a G*ΔG-luc rVSV stock at 2×10^8 IU/mL*
- 17 Remove culture media from cells transfected the previous day and gently replace with 5mL serum free DMEM containing G*ΔG-luc rVSV
- 18 Incubate for 2 hours at 37°C, 5% CO₂
- 19 Following incubation, remove media from cells and wash 5 times with 3 mL PBS
- 20 Add 8 mL fresh culture media and incubate for 24±1 hours at 37°C, 5% CO₂

Day 4: Harvest of Culture Media Containing Pseudotyped rVSV

1h

- 21 After 24 hours, collect cell culture supernatant from infected cells into a 10 mL sterile syringe.
- 22 Pass through a 0.45 μm filter attached to the syringe into a sterile 15 mL tube



- 23 Aliquot the pseudotyped virus in the appropriate volume (e.g. 200 μ L) and freeze immediately and store at -80°C . For rapid freezing, aliquots can be immersed in dry-ice.

Protocol references

Whitt, M. A. Generation of VSV pseudotypes using recombinant Δ G-VSV for studies on virus entry, identification of entry inhibitors, and immune responses to vaccines. *J. Virol. Methods* **169**, 365–374 (2010).