

Materials List for:

Generation of Recombinant Arenavirus for Vaccine Development in FDA-Approved Vero Cells

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Materials

Material and methods

Cell lines

Vero E6 (African green monkey kidney epithelial cells) are maintained in a 37 °C incubator with 5 % CO₂ in DMEM 10 % FBS 1 % PS. Cells are available from the American Type Culture Collection (ATCC, catalogue number CRL-1586).

Plasmids

All plasmids, with the exception of hpol-I L, can be grown at 37 °C, for 16-18 hr. We recommend that cultures of the hpol-I L be grown at 30 °C for 24 hr. Plasmids are prepared using a plasmid maxi kit (EZNA Fastfilter Plasmid Maxi Kit, Omega Bio-tek) following the manufacturer's recommendations and stored at -20 °C. The concentration of the purified DNA plasmid is determined by spectrophotometry at 260 nm, with purity being estimated using the 260:280 nm ratio. Preparations with 1.8-2.0 260:280 nm ratios are considered appropriate for virus rescue purposes. Additionally, plasmid concentration and purity should be confirmed with agarose gel electrophoresis.

Viruses

The described protocol for rescuing rLCMV (Armstrong 53b) and rCandid#1 can be executed under biosafety level (BSL) 2 conditions. Contaminated material, including TCS and cells should be sterilized before disposal. Rescue of other arenavirus may require higher BSL facilities so proper safety/security measures must be followed.

Tissue culture media and solutions

DMEM 10 %FBS 1 %PS: 445 ml Dulbecco's modified Eagle's medium (DMEM), 50 ml of Fetal Bovine Serum (FBS), and 5 ml of 100X Penicillin/Streptomycin (PS). Store at 4 °C. This media will be used for maintenance of Vero cells.

Infectious Media: 2-to-1 mixture of OptiMEM and DMEM 10 %FBS 1 %PS. Store at 4 °C. This media will be used during viral infections.

10X Phosphate buffered saline (PBS): 80 g of NaCl, 2 g of KCl, 11.5 g of Na₂HPO₄·7H₂O, 2 g of KH₂PO₄. Add ddH₂O up to 1 liter. Adjust pH to 7.3. Sterilize by autoclave. Store at room temperature.

1X PBS: Dilute 10X PBS 1:10 with ddH₂O. Sterilize by autoclave and store at room temperature.

2.5 % BSA: 2.5 g of BSA in 97.5 ml of 1X PBS. Store at 4 °C. This is used as a blocking solution for IFA.