

Standard Operating Procedure

I. PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to describe the procedure for producing multiple virus-like particles (VLPs) each expressing three antigens, glycoprotein (GP), a matrix protein (VP40), and a nucleoprotein (NP) of a filovirus strain in mammalian cells.

II. RESPONSIBILITY

It is the responsibility of the person performing the assay to ensure that it is carried out according to the SOP and that all relevant documents are complete and accurate.

III. MATERIALS

Appropriate viral DNA's cloned in pWRG vectors in E. coli
293 E mammalian cells
PEI, Lipofectamine or other appropriate transfection reagent.
Gibco Freestyle Medium
Hyclone HEK293 Medium

IV. EQUIPMENT

Production equipment: New Brunswick Scientific Innova 4430 or Innova 4400 Incubator
Shakers [rpms of 110 or 100 respectively]
Growth/production vessel: Corning [#431252] 3L Erlenmeyer flask, sterile, polycarbonate, non-baffled
Beckman*Coultter J6-MI centrifuge with a large rotor capable of centrifuging 2-3L per spin
Single channel pipettes
Automatic pipetman
Freezer - 70°C ($\pm 10^\circ\text{C}$)
Refrigerator, 2-8°C
Biological Safety Cabinet

V. PROCEDURE

A. Preparation of cells:

1. Determine density of 293E cell culture.
2. Centrifuge enough cells to seed 500 mls in a 3L Fernbach flask at 5×10^6 cells/ml and wash 1x with Gibco Freestyle Medium (FS).
3. After wash, re-suspend cells to 500 mls in FS medium.
4. Allow culture to equilibrate on shaker for 1-2 hours before adding DNA/PEI mixture.

B. Preparation of DNA/PEI mixture:

1. If setting multiple Fernbachs, adjust volumes and mix in one 250 ml tube then divide DNA/PEI mixture equally among the Fernbachs.
2. Add 375 ug of **each** DNA (Ebola or Marburg, VP40, GP, NP or other plasmids as indicated) to 25 ml of normal saline in 50 ml conical tube and briefly vortex.
DNA = 1.125 mg total/Fernbach flask.
3. Add 6.8 mls (6.750 mg) of PEI (approximately 1 mg/ml) to DNA and vortex for 3-5 seconds (ratio is 6 ug PEI per 1 ug DNA; ratio may vary with each lot of PEI).
4. Incubate mixture for 15 minutes at room temperature to allow complexes to form.
5. Add the DNA/PEI mixture directly to the Fernbach flasks and place on the shaker. Incubate for 4 hours at 90 rpms.

C. Post transfection:

1. Add 500 mls of HyClone HEK293 medium to the Fernbach flasks prepared above.
2. Incubate the Fernbach flasks for 72 hours then count cells and harvest supernatant.
 - a. Do not freeze supernatant, store at 4°C.