

## FIVE PLATE LYSATE

### Reagents

2% FBS / 1% P/S / DMEM.

10% FBS / 1% P/S / DMEM.

### Cells

Human Embryonic Kidney Cells – HEK 293.

Prepare 150 mm plates as follow for the cells to reach 60-70% confluency at time of infection:

Time to infection	Cells/plate
Ready next day	12 million
2 days	5 million
3 days	2 million
4 days	1.5 million

### Procedure

1. Freeze/thaw collected cells and media from 60 mm plate transfected earlier.
2. Freeze/thaw two times using a 37°C water bath and a dry ice/ethanol bath.
3. Centrifuge media lysate at 2500 rpm ( $g = 600$ ) for 10 min at 4°C.
4. Use 15.0 ml of 2% FBS / 1% P/S / DMEM media per plate for a total of 75.0 ml for (5 x 150 mm) plates.
5. Add supernatant from the media lysate to the media.
6. Remove the media from plates and add very gently 15.0 ml of the 75.0 mls media + virus to each plate.
7. Incubate at 37°C with 5% CO<sub>2</sub> until cells are at least 60% lifted from the plate.
8. Using a 20.0 ml sterile serological pipet collect the cells and media from the plates (5 x 150 mm). Transfer to two 50.0 ml conical centrifuge tubes.
9. Centrifuge at 1500 rpm ( $g = 217$ ) for 10 min at 4°C.
10. Remove the media without disturbing the cell pellet.
11. Wash the cells with 5.0 ml of 1x PBS. Very gently.
12. Centrifuge at 1500 rpm ( $g = 217$ ) for 5 min at 4°C.
13. Remove the 1x PBS without disturbing the pellet.
14. Re-suspend cells in 5.0 ml of 10.0 mM Tris.
15. Freeze cell lysate at -20°C.