Systems Virology
of Emerging Respiratory Viruses

SCL003.0P Maintenance and Plating of 2B4 cells (Amy Sims, 5/25/10)

Media:
500 mL bottle 1x MEM (Gibco cat. 11095) supplemented with
100mL Defined FBS heat inactivated (HyClone cat. SH30070.03)
7mL antibiotic/antimycotic (100x Gibco cat. 15240)

Thawing cells:
Thaw cells by placing vial in 37°C water bath and swishing around until thawed completely (approximately 2 minutes). In the tissue culture hood, add the thawed cells to a 15mL conical tube containing 9mL fresh warm media and gently resuspend cells. Spin the cells down for 4 minutes at 800 x g, remove supernatant, resuspend in 10 mL fresh media and plate in a T-25 flask.

Maintaining cells:
2B4 cells require daily feeding if you need them to grow at a more rapid pace. They will survive if you feed them every other day or every few days but their division slows down. They can take up to a week to become confluent once in a new flask even when split at 1:2, but depending on feeding schedule they can become confluent in about 4 or 5 days if fed daily.

Splitting cells:
- Remove media
- Wash cells with 7mL 1x PBS
- Add 5mL of 0.05% Trypsin and place back in 37°C incubator for about 30 minutes. Check on them frequently and tap the side of the flask against your palm to persuade the cells to release from the plate.
- Once all the cells are free resuspend in fresh media, rinse the flask a few times with the same media, and spin down (800 x g for 6 minutes). Resuspend in fresh media and plate (normally split at 1:2).

Plating for an Experiment:
- Cells are plated 48hrs before the experiment will begin
- We find that plating 1.5 x 10^6 cells per well with 3 mL of complete media for each well works best.
- Remove old media and add fresh media after 24hrs
- Infect cells on the second day after plating

Freezing Cell Stocks:
- Wash and trypsinize as above for splitting
- Resuspend cells in media and spin down
- Resuspend cells in 1x PBS and spin down
- Resuspend cells in defined heat inactivated FBS supplemented with 10% DMSO (Sigma cat. D2650) and we freeze at -140C or in liquid nitrogen