



Systems Virology of Emerging Respiratory Viruses

TCL005.0P - Maintenance and Plating of Calu-3 cells for Cytokine Treatment

Complete Media:

1. DMEM/F12 (1:1), Gibco, CAT #: 11330-032 (500mL)
2. 10% FBS (50mL)
3. 1x Antibiotic/Antimycotic (5mL), Gibco, CAT #: 15240-062

Expansion:

These cells do not grow well if plated at low density.

1. From T25 to T75 to T175 - pass at 1:2, or all of the cells into the next sized flask
2. To expand from a T175 - pass at either 1:2 or 1:3. Never dilute the cells more than 1:3.
3. Passage number should remain below 15.

Passing the cells:

1. Warm media to 37°C, warm PBS and trypsin to room temperature
2. Remove media from flask and wash with 7 mL 1x PBS and rinse the flasks for about 2 minutes, rocking them occasionally.
3. Add 2 mL (varies according to flask size) of trypsin, and incubate at 37°C. After 20 minutes the cells should be off of the flask and should begin to slide down when you pick up the flask, if not, incubate for another 10 minutes at 37°C.
4. Resuspend the cells by adding 10 mL of complete media back to each flask.
5. Add resuspended cells to 50mL conical, bringing the volume up to 50mL, pellet cells to remove all of the trypsin at 1.1g for 5 minutes.
6. Discard supernatant and resuspend in 10mL of complete media and plate cells.

Plating and cytokine treatment for 6 well plates:

1. Cells that are to be treated are to be plated in 10% FBS complete media
2. Plate at 1×10^5 cells per well
3. Washed 1 day after plating
4. Add treatment to cells 2 days after plating.
5. For mock samples follow step 3 without adding cytokine.
6. Harvest cells using the appropriate procedure depending on the assay to be performed on sample.

