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Protocol status: Working
We use this protocol and it's working

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Splitting Adherent Cell Lines

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DISCLAIMER

Timing for trypsin treatment and following splits may need to be adjusted based on the different cell types considering how adhesive they are and their growth rate.

ABSTRACT

This protocol describes how to split and maintain adherent cell lines in culture. Examples of these cells are: A549 cells, LLCMK2 cells, and MDCK cells.

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PROTOCOL integer ID: 92422

MATERIALS

Tissue Culture Medium:

DMEM	500ml
Gentamicin	500ul (50ng/ml)
NaPy	5.0ml (1mM)
L-Glutamine	5.5ml (2mM)
FBS	50ml

Filter through a 0.2um filter

INFECTION MEDIUM - SeV

DMEM	500ml
Pen/Stp	5.0ml
BSA 35%	5.0ml
NaHCO3 5%	12ml

INFECTION MEDIUM - RSV (2% FBS)

DMEM	500ml
Gentamicin	500ul (50ng/ml)
NaPy	5.0ml (1mM)
L-Glutamine	5.5ml (2mM)
FBS	10ml

Protocol

1 Splitting Adherent cells

1. Wash flask 2x with sterile PBS.
2. Add 2mL of trypsin/T75. Incubate at 37 oC for 2-3 mins or until cells are detached from the flask.
3. Add 8mL of cell culture media and pipette up and down. Transfer all the media to a 15mL tube.
4. Centrifuge at 1200 rpm for 5 min.
5. Add 2ml of cell suspension to T75 flask, then add 10ml Tissue Culture Medium. The cell will be ready for next split two days later.