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Version 1

# Splitting Adherent Cell Lines V.1

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**Protocol status:** Working

We use this protocol and it's working

**Created:** December 17, 2023

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**Protocol Integer ID:** 92422

**Keywords:** splitting adherent cell line, adherent cell lines this protocol, adherent cell lines in culture, mdck cell, adherent cell line, llcmk2 cell, a549 cell, cell, examples of these cell

## 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.

## 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            |

## Troubleshooting



## 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.