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Splitting Adherent Cell Lines V.2

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

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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.

Materials

TISSUE CULTURE MEDIUM (TCM): Filter through 0.2 µm filter

	Component	Amount	Conc. Supp.	Product information
	DMEM	500 mL		Gibco Cat. # 11965092 (#11965118-cs)
	Gentamicin	500 µL	50µg/mL	Gibco Cat #15750060 (#15750078-pk) – 50 mg/mL
	Sodium Pyruvate	5.0 mL	1mM	Corning Cat #25-000-Cl – 100mM
	L-Glutamine	5.5 mL	2 mM	Sigma Aldrich Cat #G7513 – 200mM
	FBS	50 mL	10%	

Protocol

1 **Splitting Adherent cells**

1. Wash flask 2x with sterile PBS.
2. Add 2mL of trypsin/T75. Incubate at 37ºC 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. Discard media and resuspend cells in 10mL Tissue Culture Medium
6. Add 2mL of cell suspension to T75 flask, then add 8mL Tissue Culture Medium. The cell will be ready for next split two days later.