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We are still developing and optimizing this protocol

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Passaging and plating A549 cells

Forked from Passaging and plating A549 cells

ATCC[1], Steven Walter[2]

[1]American Type Culture Collection; [2]Brigham Young University

Steven Walter

ABSTRACT

Volumes used in this protocol are for 75 cm^2flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning T-75 flasks (catalog #430641) are recommended for subculturing this product.

GUIDELINES

Most human cell lines are vulnerable to infection by the microbes on your skin, be careful to work aseptically to avoid contaminating your cultures and keep yourself safe.

MATERIALS

Complete Growth Medium containing Dulbecco's Modified Eagle's Medium (DMEM) (ATCC 30-2002). To make the complete growth medium, add the following components to the base medium: 10% Fetal Bovine Serum (heat inactivated) (ATCC 30-2020), 2mM L-glutamine (ATCC 30-2214), 1% Penicillin/Streptomycin.

Sterile 6 cm plates
Trypsin-EDTA solution
sterile serological pipettes (1mL and 10 mL)

SAFETY WARNINGS

A549 cells like most human cell lines host adenovirus and other transmissible pathogens. Only handle closed containers or denatured homogenates outside of a biosafety cabinet.

BEFORE START INSTRUCTIONS

Warm all solutions to 37 deg C and prepare biosafety cabinet by starting the blower and wiping down all surfaces with 70% ethanol. Organize your workspace in the biosafety cabinet to have all clean/fresh reagents on one side and all waste on the other. Anything you will be taking into the biosafety cabinet should also be wiped clean using 70% ethanol and paper towels

Splitting A549 in T-75

10m

1 Warm all solutions to 37 °C

2 Remove and discard culture medium, by tipping dish carefully to one side and aspirating the media without disturbing the adherent cells

3 Briefly rinse the cell layer with 1.0 mL 0.05% (w/v) Trypsin - 53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.

4 Add 2.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (about 00:10:00). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

10m

5 Add 6 mL of complete growth medium and aspirate cells by gently pipetting.

6 Remove 10 µL of cell suspension for the hemocytometer. Average the count of the four-corners and multiply by 10,000 (10^4) to get concentration (cells/mL).

7 Centrifuge 150 x g, 00:05:00 ; Remove supernatant. Resuspend in 6 mL fresh media.

5m

8 Seed new plates with the recommended amount from the table.

	A	B	C	D
		Surface Area (cm^3)	Seeding Density (10^6)	Cells at confluency (10^6)
	Plates			

	A	B	C	D
	6-well	9.6	0.3	1.2
	12-well	3.5	0.1	0.5
	96-well	0.32	0.01	0.04
	Flasks			
	T-25	25	0.7	2.8
	T-75	75	2.1	8.4
	T-175	175	4.9	23.3

9 Incubate at 37 °C for approximately 48:00:00 2d