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Passaging and plating A549 cells

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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 TEXT

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.

DISCLAIMER:

BEFORE STARTING

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

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

2 Briefly rinse the cell layer with 1 mL 0.05% (w/v) Trypsin - 53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.

3 Add 0.5mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed 5m (usually within 5 **00:05:00** to 15 minutes). 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.

4 Add 3 to 4 mL of complete growth medium and aspirate cells by gently pipetting.

5 Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C for at least an hour. Cells will adhere but not grow well until EDTA/trypsin is asperated off.

6 After cells have adhered to plate carefully aspirate away the media and feed cells new media

7 Allow cells to grow at 37°C