Passaging Mammalian Cells

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Abstract

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Protocol

Step 1.

Warm media to 37°C and bring 0.05% trypsin in PBS to room temperature.

Media may be DMEM or 1640-RPMI with 10% FBS.

Step 2.

Examine cells under a microscope to evaluate confluency and presence of cell death.

Step 3.

Aspirate out spent media.

Step 4.

Add 2 mL of trypsin to the flask and ensure that the entire adhered surface of the flask is covered with trypsin.

(Step 7 should occur within 10 minutes of adding trypsin)

Step 5.

Incubate the cells at 37°C for 5 minutes.

Step 6.

Disrupt the cells by hitting the side of the flask repeatedly, and examine under the microscope. Repeat this step until the cells have been fully detached.

Step 7.

Add 5 mL of media to the flask. Pipet up and down repeatedly and wash the side of the flask to pool all the cells at the bottom of the flask.

Step 8.

Take a sample for cell density determination and transfer the rest of the cells to a conical vial.

Step 9.

Centrifuge the suspended cells at 100 RCF for 5 minutes.

Step 10.

Aspirate out the media, minding to not disrupt the cell pellet.

Step 11.

Resuspend cells to 1 million cells/mL with media, based off of values calculated in Step 8.

Step 12.

Transfer between 1-1.2 million cells to a T-75 flask and bring the final volume to 12 mL by adding media.

Step 13.

Examine the flask under a microscope to ensure cells are present. Incubate the cells at 37ºC with 5% CO_2 for 2-3 days.