Cryo-Freeze Cell Culture

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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.

Cool Mr. Frosty to 4ºC. Mr. Frosty must contain the indicated amount of isopropanol.

Step 3.

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

Step 4.

Aspirate out spent media.

Step 5.

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 6.

Incubate the cells at 37°C for 5 minutes.

Step 7.

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 8.

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 9.

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

Step 10.

Centrifuge the suspended cells at 100 RCF for 5 minutes.

Step 11.

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

Step 12.

Resuspend cells to 3 million cells/mL with media, based off of values calculated in Step 8, with freeze media.

Freeze Media:

DMEM + DMSO (cell culture tested) to get a final solution that has 5% DMSO

or

(1640-RPMI + 10% FBS) + DMSO (cell culture tested) to get a final solution that has 5% DMSO

Step 13.

Transfer 1 mL of resuspended cells in freeze media into each cryo-vial.

Step 14.

Let the cells sit at room temperature for 15 minutes, but do not exceed 30 minutes, as cells will die.

Step 15.

Place vials into 4ºC Mr. Frosty and transfer Mr. Frosty to -80ºC freezer for 24 hours.

Step 16.

After 24 hours, transfer vials to liquid nitrogen cryo-storage.