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PhoenixSongs BIOLOGICALS

StemCell Freezing Medium And Instructions

StemCell Freezing Medium contains DMSO and is optimized for the cryopreservation of hepatic and biliary tree stem cells.

Required Supplies

1. Kubota's StemCell Growth medium (Catalog # 11001-250)
2. StemPro Accutase (LifeTech A11105-01)
3. StemCell Freezing medium for hepatic and biliary tree stem cells (Catalog # 11005-050)

Preparing hepatic and biliary tree StemCells for Cryopreservation

1. For all volumes refer to Table 1 below.
2. Working with multiple plates or flasks
    a. Only work with a stack of 4 x 100mm plates or 4 flasks at a time
    b. Minimize time out of the incubator during the process
    c. Work swiftly but carefully to minimize cell loss
3. Remove the media from the plate or flask and add cold Accutase (see table 1 for volumes) (Do not warm Accutase in the waterbath)
4. Take a plate or flask of cells immediately to the microscope so that you can observe the dissociation.

    NOTE: Do not place the plates or flasks in an incubator during the dissociation process. Accutase will loose activity if allowed to warm up to 37°C.

5. Tap the plates or flasks against the palm of your hand to dislodge the cells and when the cells are free floating, return the plates to the safety cabinet
6. Gently pipette up and down to ensure single cell suspension, then transfer cell suspension into appropriate size conical tube.
7. Wash the plate or flask with Kubota's StemCell Growth medium and add to the tube, pipetting up and down to ensure a homogenous cell suspension
8. Take an aliquot for cell counting
    a. Take 20ul cell suspension and add it to 20ul Trypan Blue
    b. Pipette up and down
    c. Load10ul of cell suspension to both counting chambers of a hemacytometer
    d. Count the cells within the center grid
    e. Calculate the total cell count

    Cell count X 10,000 X 2 X Volume = Total cell count

    Note: 10,000 is a hemacytometer constant and 2 is the Dilution factor

9. Divide the total cell count by 1.5 x 10^6 to determine the number of cryovials needed for freezing down the cells for long-term storage.
10. Pellet the cells in all the tubes by centrifugation at 500 x g for 5 minutes.
11. While the cells are in the centrifuge print the labels needed for each cryovial
12. Place one label on each cryovial.

13. After the centrifuge stops, resuspend the cell pellet in enough StemCell Freezing Medium to freeze 1.5 x 10^6/ml as calculated in step 9 above.
14. Transfer 1ml StemCell Freezing cell suspension into each cryovial
15. Transfer the cryovials into a controlled rate freezer or a styrofoam container then place the container into a -80°C freezer over night
16. Next day transfer the vials of cells into the LN2 freezer for long-term storage

Table 1. Volumes of Accutase and media

Dish/Flask Size	Growth Area (cm^2)	Accutase Volume	Media Volume
100mm	58.1	2	2
60mm	21.3	1	1
35mm	9.6	0.5	0.5
T-75	75	10	10
T-175	175	15	15
T-225	225	20	20

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