Melan-a Cell Line culture protocol - Cat # 153599

Materials required:

Material
Melan-a Cell Line (**CancerTools, Cat. No. 153599**)
Melan-a growth medium: RPMI-1640 Medium 10% Foetal Bovine Serum (FBS) 200 nM phorbol-12-myristate-13-acetate (TPA)
Cryopreservation Medium: RPMI-1640 Medium 10% FBS 10% DMSO
Phosphate buffered saline (PBS)
Accutase

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Thawing cells:

Note: The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

1. Prepare Melan-a growth medium: RPMI-1640, 10% FBS, and 200 nM phorbol-12-myristate-13-acetate (TPA), to thaw and expand the cells. The cells can grow on normal tissue culture-ware surfaces without any additional coating.
2. Remove the vial of frozen Melan-a cells from liquid nitrogen and thaw rapidly by incubating in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells. ***IMPORTANT:*** **Do not vortex the cells.**
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a microbiological safety cabinet, use a 1- or 2 mL pipette to transfer the cells into a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of Melan-a growth medium into the 15 mL conical tube. ***IMPORTANT:*** **Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.**
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles. ***IMPORTANT:*** **Do not vortex the cells.**
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove any residual cryopreservative (DMSO).
9. Resuspend the cells in 15 mL of Melan-a growth medium.
10. Transfer the cell mixture into a T75 tissue culture flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.

Subculturing cells:

Note: Melan-a cells proliferate relatively slowly. It may take several days for the cells to reach 80-85% confluency and be ready to be passaged further.

1. Do not allow the cells to grow to full confluency. Melan-a cells should be passaged at ~80-85% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80-85% confluent layer of Melan-a cells.
3. Rinse the flask with 10 mL of 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase to the flask and incubate in a 37°C incubator for 3-5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of Melan-a growth medium to the flask.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells into a 15 mL conical tube.
8. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of Melan-a growth medium into the tube and resuspend the cells thoroughly by pipetting up and down. ***IMPORTANT:*** **Do not vortex the cells.**
11. Count the number of cells using a hemocytometer.
12. Split and plate the cells into new flasks at the desired density (typical split ratio is 1:4 or 1:6).

Cryopreservation of cells:

1. Melan-a cells may be frozen in RPMI-1640 medium with 10% FBS and 10% DMSO, using a Nalgene slow freeze Mr. Frosty container.
2. Transfer Melan-a cells into liquid nitrogen for long-term storage.

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