Thaw Procedure for WEHI-231 Cells
AfCS Procedure Protocol ID PP00000116
Version 1, 10/25/02

The AfCS WEHI-231 cells were obtained from the American Type Culture Collection (ATCC; item CRL-1702; lot 2056354). The cells were thawed, expanded, and refrozen in large quantities to ensure that all subsequent experimentation in AfCS laboratories involving WEHI-231 cells is performed on cells derived from a single source. All aliquots were frozen using standard AfCS protocol (Preparation of Frozen Stocks of WEHI-231 Cells, PP00000134). Aliquots of frozen cells contain approximately $10 \times 10^6$ cells in 1 ml of freezing medium (AfCS Solution Protocol WEHI-231 freezing medium (WHFM), PS00000454). This thaw procedure will ideally yield a concentration of $3.3 \times 10^5$ WEHI-231 cells/ml.

**Thaw Procedure**

1. Suspend the tube of frozen cells in a 37 °C water bath until fully thawed. Do not leave tube in water bath unattended.
2. In a tissue culture hood, immediately pipette the thawed cell suspension into a 50-ml conical tube containing 30 ml of prewarmed WEHI-231 growth medium 1 (WHGM1, 37 °C).
3. Centrifuge the tube of cells at 400 x g for 5 min at room temperature.
4. Remove all but 10 µl of the supernatant.
5. Flick tube gently with index finger to resuspend cell pellet.
6. Add 10 ml of warm WHGM1.
7. Pipette up and down gently 2 times with a 10-ml pipette to wash cells.
8. Centrifuge at 400 x g for 5 min at room temperature.
9. Remove all but 10 µl of the supernatant.
10. Flick tube gently with index finger to resuspend cell pellet.
11. Add 30 ml of warmed WHGM1.
12. Pipette up and down gently to resuspend cells in the 30 ml of WHGM1.
13. Transfer the 30 ml of cells to a 75-cm² vented cap tissue culture flask.
14. Place flask in an incubator set to 37 °C in air with 5% CO₂.
15. Enter barcode from cryovial into appropriate WEHI GUI field.
16. Count the cells after 24 hr and split accordingly.

**Reagents and Materials**

WEHI-231 growth medium 1 (WHGM1): AfCS Solution Protocol ID PS00000434

Tissue culture flask, 75 cm² with vented cap (max. vol. 30 ml): Corning/Fisher Scientific; catalog no. 10-126-37

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