Passage Procedure for WEHI-231 Cells
AfCS Procedure Protocol ID PP00000118
Version 1, 10/24/02

Once thawed (AfCS protocol Thaw Procedure for AfCS WEHI-231 Cells, PP00000116), AfCS WEHI-231 cells are maintained at 37 °C in an atmosphere of 5% CO₂. When split (or passaged), the cells are diluted to a concentration of 1 x 10⁵ cells/ml and grown to concentrations of no greater than 8 x 10⁵ cells/ml. This procedure requires the cells to be split every 2 days.

Passage Procedure
1. Warm WEHI-231 growth medium 1 (WHGM1) for 15 to 20 min in a 37 °C water bath. (Note: all manipulations and additions to cells are done with sterile tissue culture technique.)
2. Enter the barcode of the cells into the WEHI GUI.
3. Gently pipette the cell suspension up and down in the flask to create a single cell suspension using a 10-ml pipette. This includes flushing the cell suspension over the sides of the flask on which the cells have settled during the culture period. Avoid creating bubbles
4. Remove 500 µl of the single cell suspension and transfer to a microfuge tube.
5. Centrifuge at 400 x g for 5 min.
6. Aspirate the medium.
7. Add 500 µl of magnetic cell sorting buffer (MACS buffer) and gently resuspend the cells.
8. Add 500 µl of trypan blue solution.
9. Return the flask to the incubator while counting. (Note: the change in medium for counting is necessary to keep the cells from clumping and thus obtain reproducible and accurate cell counts.)
10. Count the white and blue cells using a hemacytometer.
11. Enter the counts into the WEHI GUI.
12. Add the volume of prewarmed WHGM1, calculated by the WEHI GUI, to a new flask.
13. Remove the culture of WEHI-231 cells from the incubator.
14. Pipette the cell suspension up and down, without introducing bubbles, to resuspend the settled cells.
15. Transfer the volume of the cultured WEHI-231 cells, calculated by the WEHI GUI, to the new flask.
16. Pipette up and down 2 times to mix.
17. Place the flask in the incubator set at 37 °C containing a 5% CO₂ atmosphere.
18. Repeat steps 12 through 17 for expanding to multiple flasks.
19. Aspirate the extra cell suspension for disposal.
**Reagents and Materials**

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

Tissue culture flask, 25 cm² with vented cap (max. vol. 10 ml): Corning/Fisher Scientific; catalog no. 10-126-28

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

Tissue culture flask, 150 cm² with vented cap (max. vol. 60 ml): Corning/Fisher Scientific; catalog no. 10-126-34

Magnetic cell sorting buffer (MACS buffer): AfCS Solution Protocol ID PS00000001

Trypan blue solution: Sigma-Aldrich; catalog no. T8154

Hemacytometer: Fisher Scientific; catalog no. 02-671-5

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