The following procedure is for thawing and weekly maintenance of WEHI-231 cells. The procedure is carried out under a laboratory hood, using sterile procedures.

**Standard Growth Conditions**
2. Use a maximum volume of 10 ml medium for 25-cm² flasks; 30 ml medium for 75-cm² flasks; 60 ml medium for 150-cm² flasks; 70 ml medium for 175-cm² flasks; and 90 ml medium for 225-cm² flasks.
3. Maintain cells in a standard cell culture incubator with 5% CO₂ at 37 °C. Place flasks in the incubator flat on their broad sides, never upright.

**Cell Thawing Procedure**
1. Warm Stanford RPMI to 37 °C in a water bath.
2. Prepare a 15-ml conical tube with 10 ml of warm, sterile medium.
3. Thaw one vial of WEHI cells at 37 °C and transfer to the conical tube containing the medium.
4. Centrifuge the cells at 230 g for 5 min.
5. Remove supernatant, resuspend cell pellet in 10 ml of 37 °C medium, and transfer to a 75-cm² tissue culture flask.
6. On the following day, count the cells with a hemacytometer and check cell viability with 10 µl of 0.4% trypan blue solution for 100 µl cell suspension.
7. Split cells as described below based on viable cell count.

**Treatment of Tissue Culture Flasks Containing Cells for Counting and Splitting Cells**
   The following is a standard procedure for treatment of flasks for collecting and counting cells.
1. Warm Stanford RPMI to 37 °C in a water bath.
2. Rinse each flask 4 times by pipetting medium from the flask across the bottom using a 5-ml disposable pipette.
3. Pool all flasks that were prepared on the same date with the same cell density using a 25-ml disposable pipette.
4. Mix the contents of the flask by pipetting up and down with a 25-ml disposable pipette.
5. Count the cells using a Coulter Counter or hemacytometer and split the cells according to the weekly culture procedure (see below).
Weekly Culture Procedure

**Friday**
1. Prepare cells at $1 \times 10^4$ cells/ml in 75-cm$^2$ flasks in the number of flasks necessary based on experimental needs for the upcoming week. Assume that $1 \times 10^4$ cells/ml will yield 8 to $10 \times 10^5$ cells/ml after 72 hr in culture.

**Monday**
2. Use the flasks prepared on Friday at $1 \times 10^4$ cells/ml to prepare one set of flasks at $2 \times 10^5$ cells/ml to be used in Tuesday’s experiments.
3. Prepare the number of flasks necessary based upon the number of cells needed for Tuesday. Assume that $2 \times 10^5$ cells/ml will yield 6 to $8 \times 10^5$ cells/ml after 16 to 24 hr in culture.
4. Prepare another set of flasks at $4 \times 10^4$ cells/ml for splitting on Wednesday.

**Tuesday**
5. Use the flasks prepared on Monday at $2 \times 10^5$ cells/ml for any experiments planned for the day.
6. Use the remaining cells in the flask to prepare cells at $2 \times 10^5$ cells/ml for Wednesday’s experiments.
7. Prepare the appropriate number of flasks to generate the necessary number of cells for Wednesday’s experiments.

**Wednesday**
8. Use the flasks prepared on Tuesday at $2 \times 10^5$ cells/ml for any experiments planned for the day.
9. Use the remaining cells in the flask to prepare cells at $2 \times 10^5$ cells/ml for Thursday’s experiments.
10. Prepare enough flasks to generate the necessary number of cells for Thursday’s experiments.
11. Use cells that were prepared at $4 \times 10^4$ cells/ml on Monday to prepare a set of flasks at $4 \times 10^4$ cells/ml for splitting on Friday. If necessary, use the remaining cells in the flask to prepare cells at $2 \times 10^5$ cells/ml for additional experiments on Thursday.

**Thursday**
12. Use the flasks prepared at $2 \times 10^5$ cells/ml on Wednesday for experiments planned for the day.
13. Use the remaining cells in the flask to prepare cells at $2 \times 10^5$ in the number of flasks necessary based on experimental needs for Friday.

**Friday**
14. Use the flasks prepared at $2 \times 10^5$ cells/ml on Thursday for experiments planned for the day.
15. Use the flasks prepared at $4 \times 10^4$ cells/ml on Wednesday to prepare a set of flasks at $1 \times 10^5$ cells/ml for the following week.
Reagents and Materials
Supplemented RPMI medium 1640—Stanford (Stanford RPMI): AfCS Solution Protocol ID PS00000432

Tissue culture flask, 25 cm²: Corning Inc.; catalog no. 430639
Tissue culture flask, 75 cm²: Corning Inc.; catalog no. 430641
Tissue culture flask, 150 cm²: Corning Inc.; catalog no. 430825
Tissue culture flask, 175 cm²: Corning Inc.; catalog no. 431080
Tissue culture flask, 225 cm²: Corning Inc.; catalog no. 431082
Conical tube, 15 ml: Corning Inc.; catalog no. 430052
Hemacytometer: Fisher Scientific; catalog no. 02-671-5
Trypan blue solution, 0.4%: GIBCO/Invitrogen; catalog no. 15250061
Pipette, 5 ml: Falcon; catalog no. 357543
Pipette, 25 ml: Falcon; catalog no. 357525
Z1 Coulter Counter: Beckman Coulter; catalog no. 6605699

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