WEHI-3 cell culture and generation of conditioned medium for BMCMC culture

Materials Needed

WEHI-3 cells (myelomonocytic leukemia, macrophage-like, Balb/C Mouse cells, available from ATCC: TIB 68)
T-175 flasks, other flasks as needed
CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega Cat.# G3582): a colorimetric assay for cell viability (optional)

Medium

CMLESS Medium* (basic complete medium for growth factor-independent cells)
For 500 ml:  10% Fetal Bovine Serum, heat inactivated
             2 mM L-glutamine (add fresh as needed)
             5 x 10^{-5} M β-mercaptoethanol
             1% antibiotic/antimycotic
             bring up volume to 500 ml with DMEM
*Filter all through a 0.2 μm filter to sterilize

Cell maintenance

1. Maintain a stock of WEHI-3 cells at a concentration of 10^5 cells/ml in CMLESS medium, 37°C, 5% CO₂.
2. Check cell counts and feed cells twice weekly. For routine maintenance, spin down cells at 1000 RPM for 10 minutes, aspirate supernatant and resuspend in fresh medium at 10^5 cells/ml.

Production of conditioned medium

1. Set up T-175 flasks (as many as needed) of WEHI-3 cells at 10^5 cells/ml.
2. Incubate for 2-4 days without feeding until the cell concentration is 6 x 10^5 to 1 x 10^6 cells/ml. If the cells have not reached the target concentration by day 4, use a different flask.
3. Spin down cells at 1000 RPM for 10 minutes. Transfer the supernatant to a new tube and discard the cells.
4. Spin down supernatant again at 2500 RPM for 15 minutes.
5. Filter supernatant through at 0.45 μm filter into a sterile bottle.
6. Remove approximately 5 ml of each batch for testing (if desired), and store remainder in 200 ml aliquots at -20°C for later use.

WEHI-3 conditioned medium testing (optional)

1. For each batch of WEHI-3 conditioned medium (WEHI-3 CM) produced, prepare samples for testing by diluting 1 ml of conditioned medium in 1.5 ml CMLESS medium (40% WEHI-3 CM solution).
2. Prepare cells from the CellTiter 96® AQueous One Solution Cell Proliferation Assay by taking 2.5 x 10^6 cells in a 15 ml centrifuge tube, spun down at 800 RPM
for 8 minutes at room temperature. Resuspend cells in 5 ml CMLESS medium so that final cell concentration is $5 \times 10^5$ cells/ml.

3. For each batch tested, add 50 $\mu$l of 40% WEHI-3 CM into a well of a 96 well flat-bottomed plate. Place plate in incubator at 37°C, 5% CO$_2$ to equilibrate for 1 hour.

4. Add 50 $\mu$l of CellTiter/CMLESS cell suspension from step 2 to each well and shake gently to mix. The final cell concentration should be $2.5 \times 10^4$ cells/well.

5. Incubate the plate at 37°C, 5% CO$_2$ for 72 hours.

6. After incubation, add 20 $\mu$l of AQ$_{ueous}$ One Solution reagent into each well then incubate for 2-4 hours.

7. Record absorbance at 490 nm in a 96 well plate reader.