How to make WEHI conditioned medium

Follow this schedule precisely. It is critical that the seeding density of the large T150 or T165 flasks not be too high because if that is the case, you will need to harvest the conditioned media before the titer reaches maximal level.

1. thaw WEHI cells and seed into 10 ml of RPMI/10% FBS. WEHI cells partially attach. WEHI cells are ready to be split when the adherent population covers most of the flask surface. The cells should still be healthy looking. This takes 2-3 days from time of thaw. The cells need to be gently scraped off the bottom of the flask and both attached and suspended cells are collected.
2. split 1:10 (1 ml of culture) once into T75 flasks with 30 to 40 ml of media (final volume depends on the volume of WEHI you want to make), They should be ready for transfer in 2 days.
3. split 1:20 (1.5 ml of 30 ml culture or 2 ml of 40 ml culture) into T162 flasks (100-150 ml of medium) - use RMPI/15% FBS with 1:1000 gentamicin. Pipet media in - don't pour.
4. leave for 10-14 days (media will turn yellow) but cells should still be viable even after 10 days.
5. transfer by carefully pouring into 200 ml NUNC conicals. Use a pipet to retrieve the last bit of conditioned medium
6. Spin at 2000 rpm for 15-20 minutes. It is essential to pellet the cells before filtering.
7. Filter through 500 ml bottle top units (0.2 or 0.45 μm) into sterile bottles reserved for tissue culture only (clean only with diluted bleach, never with detergent). Aliquot 45 ml (it expands on freezing) per conical and freeze at -20 deg. Never re-freeze after thawing. Keep working tube at 4 deg.
8. Always titer a new batch against the previous batch.

How to titer WEHI-CM on 3D cells

Indicator cell line: any 32D cell derivative

WEHI: Old WEHI and NEW WEHI (at 1%, 5%, 10%)

Supplies: 96 well plate

Seeding density: 2 x 10^4/ml

Counting (hemocytometer) frequency: daily for 3 – 4 days

Number of cells needed:

200 ul per well

4 days in duplicate

3 WEHI concentrations

2 WEHI lots

- 5 x 10^4 x 0.2 ml x 2 x 4 x 3 x 2 = 4.8 x 10^5 cells total (minimum)

Test media : 2X concentrated. Need 100 ul per well. Make up 1.2 ml in eppendorfs.

1% WEHI in RPMI + 10% FBS (make 2 %)

5% WEHI in RPMI + 10% FBS (make 10%)

10% WEHI in RPMI + 10% FBS (make 20%)

Protocol

1. Count cells in duplicate. Aliquot out 8 x 10^5 cells (to minimize loss and pipetting errors)
2. Dilute cells into HBSS
3. Spin out. Wash x 2 with HBSS
4. Aspirate as much as possible, use pipet tip if needed.
5. Resuspend cell pellet in 8 ml of RPMI + 10% FBS. This gives 2X concentrated cell density (8 x 10^5 cells in 8 ml is 1 x 10^5/ml)
6. Aliquot 100 ul per well
7. Add 100 ul of 2X test media
8. Incubate at 37 deg
9. Daily cell counts (in duplicate) using a hemocytometer. Add 40 ul of Trypan Blue directly to well, pipet up and down.