Barton Lab Protocol

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How to grow J558L (GMCSF producing mouse macrophages)

First, recover frozen cells in 20% IMDM, no G418. Grow them in T25 Nunc flask, in 4mls. When cell line is established, add G418 to media (1mg/ml) and cut down FCS to 5%. At this point expand them into a T175 Nunc flask, grow the cells in about 175 mls (200mls max).

Once the cells are fairly dense in the T175 flask (1 x 10^6/ ml), you can expand them into four T175 flasks. Hit the flask hard to get all the cells floating, you can also pipet them off the flask wall. If you need to freeze down a few vials you should take sample of the cells to a separate conical tube to freeze down. Centrifuge for 5 mins, 1200 rpm. I used 4 conicals, each with ~50ml of the supernatant because the T175 flask had about 175 – 200ml in it.

Discard the supernatant, wash cells in 20mls of 5% IMDM- no G418, centrifuge again.

If you would like to use Roller bottle:
Discard supernatant, pick up pellet in 500ml (4x10^5/ml) in 5% IMDM no G418 into a 1000ml roller bottle. Count cells 2 days later- should be at least double. When cell count is 1 x 10^6/ml add 700ml more media. Let the cells grow till they start to die. The media will also turn yellow.

If you would like to use T175 Nunc flasks:
Discard the supernatant and resuspend the pellet in 50mls. Transfer that 50mls to a T175 Nunc flask and fill up to 175ml of 5% IMDM no G418. Do the same for the 3 other conicals.

When the media is yellow and the cells are dying out, harvest and centrifuge down the cells. This should take about a week. Filter the supernatant and titrate to test for which concentration to use the GMCSF in. Freeze (-80) down the supernatant in 10ml aliquots. Use snap cap tubes.