L Cell Conditioned Medium (LCCM)

Cells: L-929 cells (https://www.atcc.org/products/all/CCL-1.aspx#documentation).

Culture media: DMEM, Penicillin/Streptomycin, and 10% heat-inactivated FBS.

The cells are cultured on tissue culture plates, and trypsin is used for detaching the cells. The regimen must be followed to the letter, as changing the split ratio, date of feeding, or date of collection may alter experimental outcomes. In particular, if these parameters are changed, the concentration of M-CSF (CSF1) may be different, and the L929 cells may produce metabolic byproducts that affect macrophage differentiation.

1) Split confluent L929 cells on Monday at a ratio of 1:10.
2) Feed the cells on Wednesday.
3) Collect media on Friday, which is ready for use in differentiating mouse bone marrow cells into macrophages.
    a. We replace the media on a few plates on Friday and discard the rest.
    b. Of the ones we replace the media on Friday, we keep these over the weekend to split the following Monday.