KITL/FLT3L culture FAQ by Feiya Ou

Based on frequent inquiries regarding the KITL/FLT3L protocol described in https://doi.org/10.1002/eji.202250201 (Ou et al, *Eur J Immunol*, 2023), I have compiled answers to the most common questions:

Plating density and medium volume

I recommend plating 6.25 k ACK-lysed BM cells per mL medium. Practically, this corresponds to:

- 500 k cells in 0.8 mL medium in a 24-well
- 2.5 M cells in 4 mL medium in a 6-well
- Adjust accordingly for other formats

Recombinant KITL (SCF) and FLT3L

- KITL (SCF): Recombinant KITL (SCF) works (see Supporting Information Figure 1C). I recommend using 200 ng/mL Mouse SCF Recombinant Protein (PeproTech)
- FLT3L: Although I have not tested recombinant FLT3L in the KITL/FLT3L system, 100 ng/mL recombinant FLT3L (PeproTech) is widely used in FLT3L-alone cultures and is very likely effective

Sources of conditioned media

- Human Flt3L-Fc conditioned media: J558L Cell line from Dr. Marina Cella (mcella@wustl.edu)
- Murine KITL conditioned media: CHO Cell line from Dr. Kyunghee Choi (kchoi@wustl.edu)

  If requesting these cell lines, please explain your intended use and include Ken (kmurphy@wustl.edu) in the correspondence.

  However, I recommend starting with recombinant proteins for pilot experiments, as they are more readily accessible.

Medium changes

Two approaches work equally well in my hands:

- In-plate spin: Briefly centrifuge the culture plate, carefully aspirate the old medium without tilting the plate, and add fresh medium
- Tube transfer: Transfer culture contents into a conical tube, centrifuge, discard old medium, resuspend in fresh medium, and return cells to the original well

  Note: There is no need to expand culture volume throughout the culture period.

Duration of the culture for sorting cDC1s

I recommend a total of 9-10 days of culture to ensure that all cDC1s have fully upregulated XCR1

Expected yield of cDC1s per mouse

- On average, the KITL/FLT3L protocol generates ~0.5 cDC1s per input ACK-lysed BM cell (see Figure 1C)
- From one mouse, I typically obtain 50–100 M BM cells, which yields ~25-50 M cDC1s
- Yield varies depending on conditions (e.g., recombinant proteins vs. conditioned media), but a conservative expectation is >= 10 M cDC1s per mouse. *Note that this is still a huge number of cells, usually more than sufficient for most downstream experiments*

Removing contaminating macrophages

Contaminating macrophages can be eliminated by gating out Ly-6C$^+$ cells during cell sorting. This is only necessary when sorting cDC2s and is not required when sorting cDC1s