Pullen Lab Protocol for IgE-mediated Crosslinking of BMMC

The following procedure is standard for mouse BMMC. MC cultures from other sources might need to be optimized; see literature from the Ryan lab for starting points if this is the case (*e.g.* Qayum AA, *et al.* (2016) *The Journal of Immunology*).

Materials:

- BMMC at least four weeks in culture
- Tissue culture treated sterile well plates (usually we use 6-well, but this will depend on the amount of source material available… sometimes need to scale to 24-well if there are not many cells)
- IgE, clone C38-2 κ isotype, cat. 557079 from BD Biosciences
- TNP-KLH, cat. sc-396496 from Santa Cruz Biotechnology (this is the actual immunogen for the IgE), or DNP-HSA cat. A6661 from Sigma.
- cRPMI supplemented with IL-3
- Micropipettes and tips
- Counting device (slides, counter, etc.)
- Trypan blue

Procedure:

1. Determine how many cells you need, and separate them from the general population into a new flask or well plate. Add IgE at a 1:1000 dilution (0.5μg/mL final concentration). Incubate overnight in the CO_2 incubator.
2. Gather cells and centrifuge 8.5min at 1500rpm.
3. Discard supernatant, be careful not to disturb the cell pellet!
4. Rinse with SCF-free medium or DPBS, then repeat steps 2 & 3.
5. Resuspend cells in cRPMI containing IL-3; 0.5-1 million cells/mL.
6. Split cells into various wells needed for different conditions. Bear in mind that for every crosslink (XL) you do, you should have a non-XL control in a separate well. Ideally you would do this with a minimum of 3mL in each well of a 6-well plate. This is assuming there are enough. You can, however, scale down to 24-well plates (0.5mL-1mL each well) if you do not have a lot of cells or if you have many conditions.
7. To your XL wells add the antigen (either TNP or DNP) to a final concentration of 0.3μg/mL, then incubate overnight.
8. Collect cells, and make sure all conditions are kept separate and labeled.
9. Count the cells and RECORD. This number will be very important later for normalizing cytokine concentrations.
10. Centrifuge as in step 2.
11. SAVE THE SUPERNATANTS – these contain your cytokines. If you are interested in the proteins or RNA in the cells, then proceed to extraction from the pellets using the respective protocol, otherwise discard the pellet.
12. Store supernatants -80ºC until you are ready to measure cytokines (*e.g.* with ELISA).