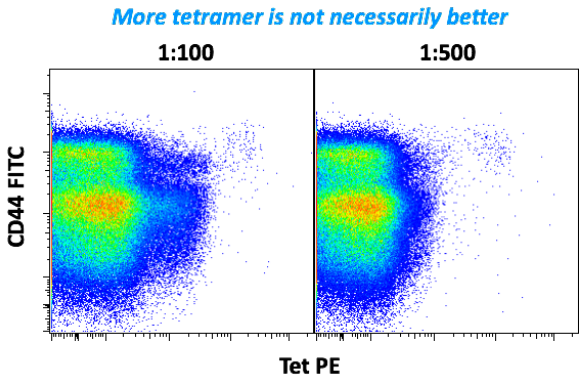
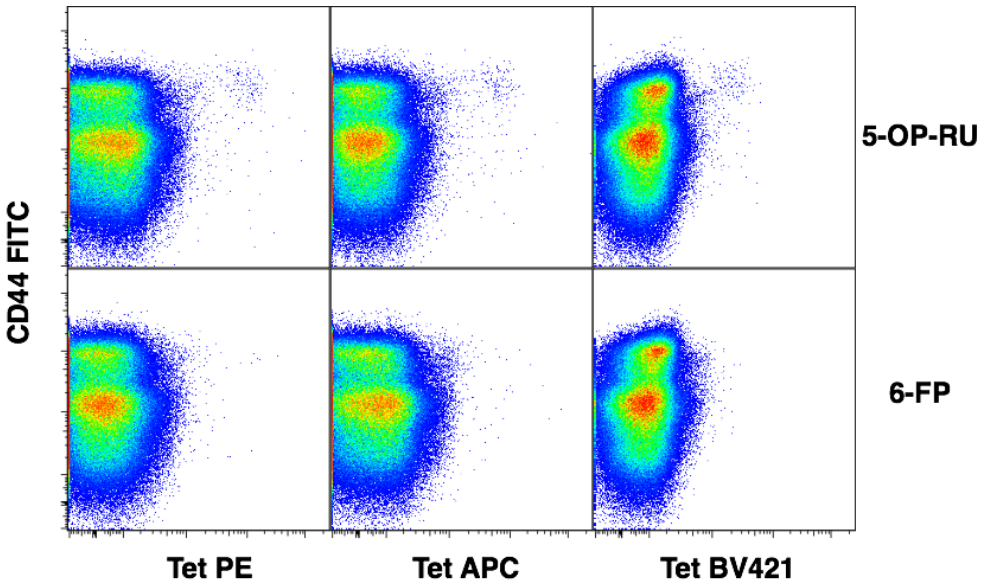
# Mouse MR1 tetramer staining

Identification of MAIT cells with MR1 tetramers was described in "T-cell activation by transitory neo-antigens derived from distinct microbial pathways." Corbett AJ, Eckle SB, Birkinshaw RW, Liu L, Patel O, Mahony J, Chen Z, Reantragoon R, Meehan B, Cao H, Williamson NA, Strugnell RA, Van Sinderen D, Mak JY, Fairlie DP, Kjer-Nielsen L, Rossjohn J, McCluskey J. (2014). Nature. 509, 361-5. DOI: http://dx.doi.org/10.1038/nature13160 PMID: 24695216. Please cite this paper if you publish using these tetramers.

Mouse MR1 tetramers are loaded with 5-A-RU in the presence of methylglyoxal, resulting in the activating ligand 5-OP-RU. We are providing 6-FP loaded MR1 tetramers as a negative control. Unlike humans, MAIT cells are rare in most lab strains of mice, ranging from <0.1% in spleen to 0.5% in liver and lung. Careful attention to the staining protocol is required for good results.

- We only provide mouse MR1 with the brightest fluorochromes (PE, APC and BV421).
- Clients should titrate the tetramers, but 1:500 is a good starting point. Using higher concentrations is wasteful and will increase background staining.
- Remove aggregates from the tetramer solution to reduce background staining. Make a working dilution, spin at top speed in a microfuge for 10 min, then add to cells.
- Collect a large number of events (at least 1-2 million).
- Stain for no more than 30 min at room temp. Costain with tetramer and other surface stains, including anti-TCRβ (H57-597), which might cluster TCRs and improve staining.
- Also include a "dump" channel to gate out cells that non-specifically bind tetramers (B220 and F4/80 are good choices).
- Carefully compare staining with the 5-OP-RU and 6-FP tetramers. You can request them with different fluorochromes to include them in the same tube.




C57BL/6 spleen cells were stained as above, tetramers added at 1:500. Cells were gated on $TCR\beta^{+}B220^{-}F4/80^{-}$ lymphocytes.

Please contact Rick Willis, Technical Director (richard.willis@emory.edu, 404-727-7215), with any questions about the reagents. Thanks to Dale Godfrey and Hui-Fern Koay (University of Melbourne) for suggestions on the staining protocol.