Coupling the biotinylated monomers (monomer-bio) to streptavidin-PE (SA-PE)

Notes:
It’s best to couple the monomer-biotin with SA-PE the day before the staining procedure, since the tetramer form is not very stable. (I don’t use tetramer that’s more than a day or two old).

1. Determine the amount of tetramer you need for your expt.
2. Add the amount of FACS buffer you need to add to get a monomer-bio concentration of 250ug/ml (which corresponds to 5 uM).
3. Calculate the amount of SA-PE you need to add to the monomer-bio: you should do the coupling at 5 monomer-bio : 1 SA-PE. The concentration of SA-PE from Biosource is 4.1uM, and you need it to be 1uM in the final volume.
4. Add SA-PE to the diluted monomer-bio one tenth of the SA-PE volume at a time, vortexing and incubating in the dark for 10min after each addition.
5. Incubate overnight in the fridge.
6. The next day, dilute it to 200ug/ml of monomer-bio. Then dilute further for step 4 of the Staining Procedure.

Materials:
Streptavidin-PE – MUST use the one from Biosource, cat#SNN1007
Tetramer FACS buffer: 2% FCS, 0.04% sodium azide (note lower azide concentration), 25mM HEPES buffer, in PBS or DMEM without phenol red.

Tetramer Staining

General procedures:
- keep cells on ice except for the neutravidin/FcR blocking step and the tetramer incubation.
- Do the staining in conical polypropylene FACS tubes (not plates), and when resuspending cells (e.g. after spinning), do so by pipetting up and down (not vortexing, etc).

1. Make single cell suspension of your cells. Lyse excess RBCs.
2. Block: 1ul of neutravidin + 1ul of FcR block in 100ul of FACS buffer. Incubate 1 hour at RT.
3. Wash with 1ml of FACS buffer.
4. Immediately resuspend in FACS buffer containing tetramer (10ug/ml of BDC tetramer, 50ug/ml of KRN tetramer). Total volume 100ul. Incubate 1 hour RT in the dark.
5. Wash with 1ml FACS buffer.
6. Stain with other surface staining Abs in the usual way (**note that you cannot use any biotinylated Abs, since the tetramer contains biotin!**)
7. Wash
8. Resuspend for analysis. Analyze the cells on the same day of staining (don’t fix). Immediately before acquisition, add 0.1 – 1ug/ml of Hoescht.

Notes:
- tetramers can give a lot of background staining, so you should always gate out B220+ cells and dead cells (i.e. Hoescht negative cells). Also stain with CD4 (I use PETR).
- KRN tetramer (GPI/g7) stains in a tight smear. BDC tetramer (2.5mi/g7) gives better separation.
- B6 cells should show very minimal tetramer staining (<1%)

Materials:
24G2 Fc block
Neutravidin (Unlabelled streptavidin) 5mg/ml stock. MolecularProbes cat# A-2666.
Tetramer FACS buffer: 2% FCS, 0.04% sodium azide (note lower azide concentration), 25mM HEPES buffer, in PBS or DMEM without phenol red.