Creating SA-PE*AF647 Decoy Reagent From Justin Taylor Lab, FHCRC

Materials

SA-PE (Prozyme, PJRS25)
AF647 labeling kit (Molecular Probes, A20173)
Amicon Ultra centrifugation filters (100k cut-off)

Procedure

1. From the SA-PE stock, take 250 ul and wash in 15ml of PBS. Concentrate SA-PE in the Amicon Ultra centrifugation filter. Add 15ml of PBS to washed SA-PE and wash again, concentrating the SA-PE to 250 ul.
2. Resuspend washed SA-PE to 500 ul in PBS, and add 50 ul of 1 M Sodium Bicarbonate.
3. Allow reactive dye to warm to room temperature, and transfer SA-PE to the reaction vial. Cap the vial and invert a few times to fully dissolve the dye. Stir the reaction vial for 1 h at room temperature.
4. Pipet SA-PE*AF647 into new Amicon Ultra (100k) and wash with PBS to remove excess AF647. Repeat washing 1-2 times. The SA-PE*AF647 will not flow through and will retain a purple color while the media in the bottom of the tube will contain free AF647 and will be blue. By the second wash the media in the bottom of the tube should be clear.
5. Collect completed product and calculate concentration of PE, AF647 and SA.
6. Measure the OD566, OD650 (try 1:10 dilution) and calculate the PE concentration. The extinction coefficient for PE is 1.96 cm-1 uM-1
   Concentration PE (uM)= OD566 x dilution factor/ 1.96
   Concentration AF647 (uM)= OD650 x dilution factor/ 0.239
7. To calculate the concentration of tetramer, correct for the ratio of SA:PE by taking the ratio of SA:PE given by the manufacturer and multiplying this by the PE concentration. This is now your real concentration of non-specific tetramer.
8. Dilute an aliquot for a working stock, at a concentration of 1.0 uM and store at 4°C.
9. Load with Biotin or irrelevant protein prior to use. It is critical that if your protein of interest has something the native protein wouldn't (like a HIS tag), the irrelevant protein must also contain this added epitope. To load, I generally just add an excess of free biotin or biotinylated irrelevant protein without a purification step after.