Comparison of CFSE and PKH26 with CellVue™ Claret, A New 675nm-emitting Proliferation Dye

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GOAL
Cell tracking dyes such as PKH26 and CFSE have proven useful in numerous applications including assessment of cell proliferation. We sought to determine whether CellVue™ Claret (formerly PTIR289), a far-red fluorescent membrane intercalating dye, could be used as an alternative to PKH26 and CFSE in multicolor proliferation studies on a standard 2 laser/4 color BD FACSCalibur.

EXPERIMENTAL STRATEGY
1) Identify optimized staining conditions for CellVue Claret (not shown). Concentrations selected for this study: a) Glutaredyl groups: can attach to unstained cells at TD. b) Did not alter viability or response kinetics of lymphocyte subsets in ex vivo use; unstained cells were indistinguishable from cells stained with CellVue Claret.
2) Optimize gating strategy for lymphocyte subpopulation analysis by incorporating a viability dye in addition to light scatter gating (Figure 1A and 1B).

NOTE: Previous experience in our laboratory had found that use of light scatter gating alone resulted in inclusion of some dead cells, which exhibited reduced proliferation dye intensity due to loss of membrane and membrane cytoplasm. This led to overestimation of extent of cell proliferation, with results being most pronounced in lymphocyte subsets present at low frequency.

3) Compare lymphocyte viability (Figures 2A and 3A), proliferative fractions (Figures 2B and 3B), precursor frequencies (Figures 2C and 3C), and 7 cell proliferation profiles (Figures 4 and 5) obtained using CellVue Claret, PKH26 or CFSE dye diction to monitor proliferation.

NOTE: 3-way dye comparisons were carried out in 6 independent studies using 3 different donors, three of whom exhibited strong proliferative responses to anti-CD3+IL-2 stimulation and two of whom exhibited moderate proliferative responses. Data shown (Donors 4 and 5) are representative of results for strong responders.

DONOR 5

Figure 5. Proliferation Profiles (96h)

CONCLUSIONS
• CellVue Claret does not appear to alter lymphocyte subset frequencies (Figure 1), viabilities (Figures 2A and 3A), or kinetics of proliferative responses to anti-CD3+IL-2 stimulation (Figures 2B, 2C, 3B, 3C, 4 and 5), whether a donor was a strong or moderate responder (data not shown).

• The ability to see discrete peaks in the proliferation profile does not appear to be required for accurate proliferation analysis. Proliferative fractions and precursor frequencies found using CellVue Claret are very similar to those of CFSE and PKH26 despite its somewhat broader background CVs (Figures 4 and 5).

• CellVue Claret thus offers a useful alternative to CFSE and PKH26 for multicolor cell tracking and/or proliferation protocols, allowing broader range of choices for antibodies and/or genetic markers (e.g. GFP and dsRed).

FUTURE PLANS
Combine CellVue Claret with a 2nd cell tracking dye to resolve proliferative responses in donor and responder lymphocyte subpopulations present in a two way mixed lymphocyte reaction.