Superior live-cell tracing, generation after generation

The CellTrace™ Far Red Cell Proliferation Kit.

Much of our current knowledge of the immune system derives from the ability to initiate an *ex vivo* immune response in isolated T lymphocytes, triggering their proliferation. By using a fluorescent label that is divided evenly between two daughter cells following cell division, researchers can detect and quantify this cell proliferation through multiple generations (Figure 1).

The dyes in our CellTrace™ Cell Proliferation Kits (Table 1) easily cross the plasma membrane and covalently bind inside cells, where the stable, well-retained fluorescent label offers a consistent and reliable fluorescent signal without affecting morphology or physiology. Importantly, these dyes produce fluorescent staining with very little variation between cells within a generation, allowing each generation to be reliably distinguished. The intense fluorescent staining provided by the CellTrace™ dyes permits the visualization of proliferating cells through 6 to 10 generations, even after several days in a cell culture environment or following fixation.

Novel red laser–excitable cell tracing reagent

We have recently introduced CellTrace™ Far Red dye, a red laser–excitable dye with narrow far-red emission (excitation/emission ~630/661 nm). These spectral characteristics make CellTrace™ Far Red dye ideal for multiplexing with commonly used 488 nm–excitable fluorophores (e.g., Alexa Fluor® 488 dye and fluorescein) and fluorescent proteins (e.g., GFP , see Figure 2, and R-PE), as well as with commonly used 405 nm–excitable fluorophores (e.g., Pacific Blue™ and Pacific Orange™ dyes). When compared with the conventional CellTrace™ Far Red DDAO-SE dye, CellTrace™ Far Red dye clearly outperformed the older dye, enabling superior generational tracing (Figure 3).

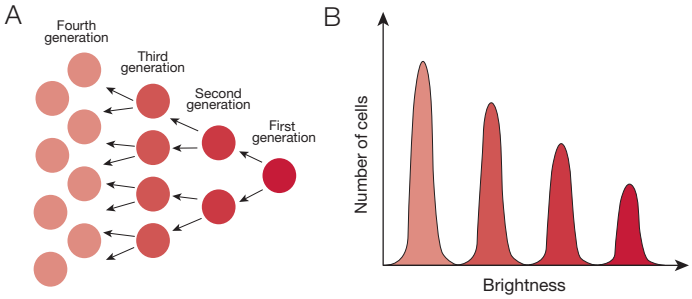

Figure 1. Bright, well-retained dye that partitions evenly between daughter cells facilitates proliferation analysis. (A) Illustration of proliferation analysis by dye dilution. **(B)** Flow cytometric analysis reveals a bright, homogeneous fluorescent signal from the initial population of cells. Subsequent cell divisions result in larger numbers of cells, each with half the fluorescence intensity of its parent cell.

Maximal flexibility in panel design

The advancement of flow cytometer multiplexing capabilities makes it imperative that researchers have flexibility in choosing functional dyes that are compatible with commonly used fluorophores and fluorescent proteins, without compromising their results. The three CellTrace™ Cell Proliferation Kits (Violet, CFSE, and Far Red) offer this flexibility to researchers who want to include generational analysis in their experiments. Not only do these kits provide three distinct detection colors, but each signal is efficiently generated with one of the three most common excitation sources for flow cytometry: the 405, 488, and 633/635 nm lasers (Figure 4, Table 1).

All three CellTrace™ Cell Proliferation Kits can be used in multicolor flow cytometry experiments to precisely track the proliferation of cultured cells while collecting other data. Panel design is critical in these multicolor experiments; to learn more about designing an effective multiplex panel, see the description of the webinar "Basics of multicolor flow cytometry panel design" on page 2.

Explore the complete set of CellTrace™ Cell Proliferation Kits

Learn more about these three CellTrace™ Cell Proliferation Kits and select the CellTrace™ dye that is right for your experiments at **lifetechnologies.com/celltracebp70**. ■

Table 1. Selection guide for CellTrace™ Cell Proliferation Kits.

CellTrace™ Cell Proliferation Kit	Laser type	Ex/Em *	Cat. No.
CellTrace™ Violet	Violet	405/450	C34557
CellTrace™ CFSE	Blue	492/517	C34554
CellTrace™ Far Red	Red	630/661	C34564

* Fluorescence excitation (Ex) and emission (Em) maxima, in nm. All kits provide sufficient reagents for 180 assays.

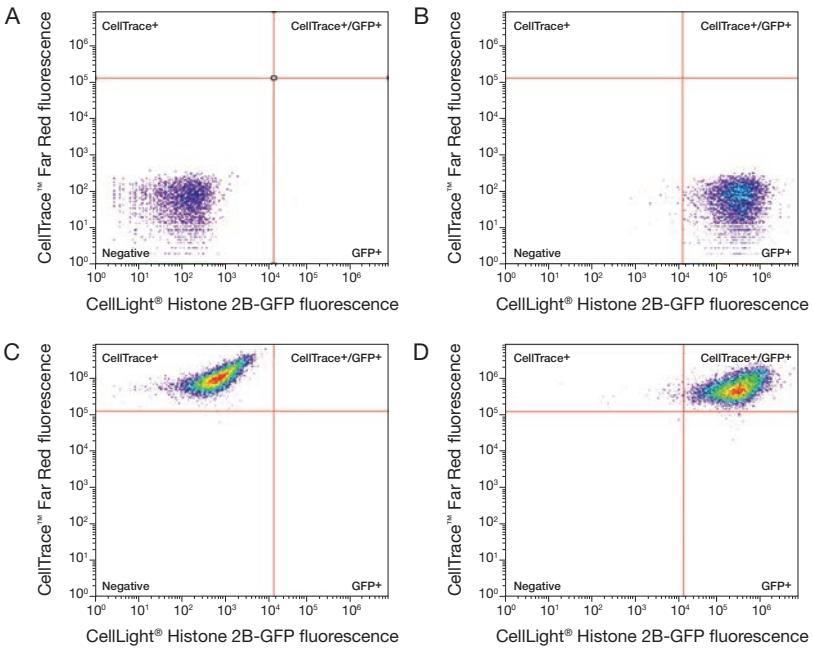

Figure 2. Live-cell multiplexing of CellTrace™ Far Red dye and CellLight® Histone 2B-GFP. U2OS cells were transduced with CellLight® Histone 2B-GFP (Cat. No. C10594). After 24 hr, cells were trypsinized, labeled with 5 µM CellTrace™ Far Red reagent (provided in the CellTrace™ Far Red Cell Proliferation Kit, Cat. No. C34564), and then analyzed using the Attune® Acoustic Focusing Cytometer with 638 nm excitation and a 660/20 nm bandpass emission filter: **(A)** unstained cells; **(B)** cells labeled with CellLight® Histone 2B-GFP; **(C)** cells stained with CellTrace™ Far Red reagent; **(D)** cells co-labeled with CellLight® Histone 2B-GFP and CellTrace™ Far Red reagent.

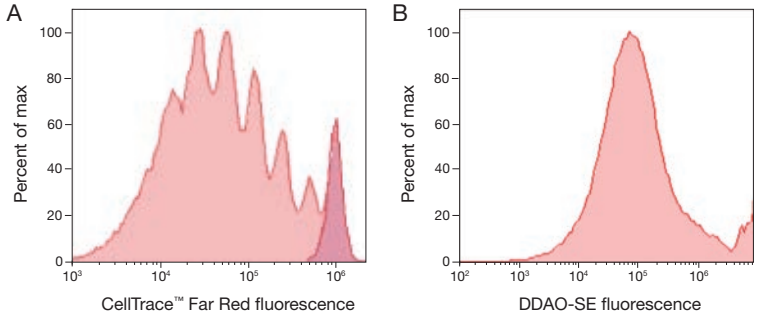

Figure 3. Comparison of generational tracing using CellTrace™ Far Red reagent and DDAO-SE. (A) Cell proliferation was followed for 7 generations using the CellTrace™ Far Red Cell Proliferation Kit (Cat. No. C34564). Human T lymphocytes were harvested and stained with CellTrace™ Far Red reagent prior to stimulation with anti-human CD3 (Cat. No. MHCD0300) for 5 days. **(B)** Peripheral blood mononuclear cells were isolated from human whole blood, stained with DDAO-SE (Cat. No. C34553), stimulated, and cultured for 5 days. Analyses of both reagents were completed using the Attune® Acoustic Focusing Cytometer with 638 nm excitation and a 660/20 nm bandpass emission filter.

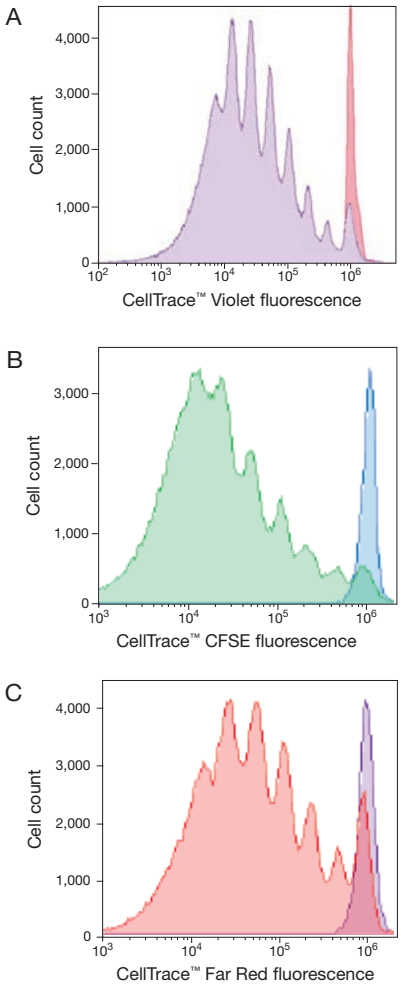

Figure 4. Generational tracing using CellTrace™ reagents. (A) Cell proliferation was followed for 8 generations using CellTrace™ Violet reagent. Human peripheral blood mononuclear cells were harvested and stained with CellTrace™ Violet reagent prior to stimulation with mouse anti-human CD3 antibody (Cat. No. MHCD0300) and interleukin-2 recombinant human protein (Cat. No. PHC0027) for 7 days. The discrete peaks in this histogram represent successive generations of live, CD4-positive cells. Analysis was completed using the Attune® Acoustic Focusing Cytometer with 405 nm excitation and a 450/40 nm bandpass emission filter. The unstimulated parent generation is indicated in red. In **(B)** and **(C)**, cell proliferation was followed for 7 generations using similar methods, but with CellTrace™ CFSE and CellTrace™ Far Red reagents, respectively. Analysis was completed using the Attune® cytometer with **(B)** 488 nm excitation and a 530/30 nm bandpass emission filter or **(C)** 638 nm excitation and a 660/20 nm bandpass emission filter.