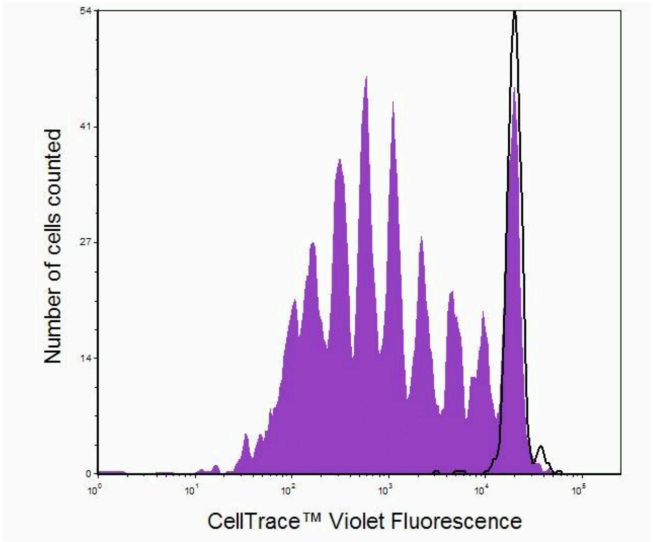
# Cell Trace Violet (CTV)

Thermo Fischer Scientific
Catalog No. C34557

CellTrace™ Violet Cell Proliferation Kit is used for *in vitro* and *in vivo* labeling of cells to trace multiple generations using dye dilution by flow cytometry. Successful proliferation analysis by dye dilution (see figure below) requires an extremely bright dye to distinguish fluorescently labeled cells from auto-fluorescence after several cell divisions. The intense fluorescent staining provided by CellTrace™ Violet dye enables the visualization of ten or more generations of proliferating cells before the signal is overwhelmed by intrinsic cellular auto-fluorescence.




**Human peripheral blood lymphocytes were harvested and stained with CellTrace™ Violet and analyzed by flow cytometry**
Human peripheral blood lymphocytes were harvested and stained with CellTrace™ Violet. The violet peaks represent successive generations of cells stimulated with mouse-anti human CD3 and Interleukin-2 and grown in culture for 7 days. The peak outlined in black represents cells that were grown in culture for 7 days with no stimulus.

## Staining Protocol

Use 15 ml tubes for the staining.

1. Resuspend cell as such: 1*106 cells/mL PBS 1X.
2. Resuspend cell trace violet vial with 20µL of DMSO. Final concentration: 5mM.
3. Add 0.5 µL/mL of resuspended cell trace violet to the cell suspension.
4. Incubate 20min at 37°C, protected from light.
5. Washing step: Add 10mL of PBS1X + FCS 5%.

6. Centrifuge at 1500 rpm, room temperature during 5min.
7. Repeat the washing step once more.
8. Resuspend the cell in the appropriate medium for culture and/or stimulation.
9. Use a small fraction of the CTV stained cells to set-up the settings of the experiment at the cytometer (detection channel: **405-450/50**). For that, resuspend the cells in 200uL of PBS1X + FCS2%, acquire and record as Day 0.
10. Acquire your stimulated cells (days after) in the same experiment where you saved the day 0 sample. The PMT values of the detection channel for CTV should not be modified.

*Do not forget to add a cell viability dye to exclude dead cells.