GeneCopoeia™
Expressway to Discovery

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# Product Information

## Cell-ID™ Violet Cell Proliferation Kit

**Catalog Number: A002**

**Unit Size: 200 assays**

**Kit Components**

**Component A: 10 vials Cell-ID™ Violet**
**Component B: 500 µL anhydrous DMSO**

**Storage upon receipt:**
- -20°C
- Protect from light

**Ex/Em:** 405/450 nm (hydrolyzed product)

## Product Description

The Cell-ID™ Violet Cell Proliferation Kit provides convenient single-use vials for cell labeling. The Cell-ID™ Violet reagent easily diffuses into cells and bind covalently to intracellular amines, resulting in stable, well-retained fluorescent staining that can be fixed with aldehyde fixatives. Excess unconjugated reagent passively diffuses to the extracellular medium, where it can be quenched with complete media and washed away.

The approximate excitation and emission peaks of Cell-ID™ Violet reagent after hydrolysis are 405 nm and 450 nm, respectively. Cells labeled with Cell-ID™ Violet reagent can be visualized by fluorescence microscopy using standard DAPI filter sets or analyzed by flow cytometry in an instrument equipped with a 405 nm excitation source.

## Materials Required but Not Provided

- PBS or other suitable buffer
- Aldehyde-containing fixative

## Protocols

The following protocols are for use as general guidelines. Because of differences in cell types and culture conditions, optimization of the protocols is required. We recommend testing Cell-ID™ Violet reagent at a starting concentration 1-10 µM. A dye concentration of 5–10 µM is recommended for tracking five or more generations, while 1–2 µM may be sufficient for tracking less than five generations. Microscopy experiments may require up to five–fold higher concentration than that used for flow cytometry. Use the lowest concentration of dye that yields good fluorescence signal to minimize cellular toxicity. For cell division tracking, we recommend analyzing a sample of freshly labeled cells that have not allowed to divide after labeling to observe the location and density of the fluorescent peak representing the undivided cell population.

**Note:** Cell-ID™ Violet dye reacts with amine groups and should not be used with amine-containing buffers such as Tris-based buffers, or with poly-lysine coated culture vessels or slides.

## Cell-ID™ Violet Stock Solution Preparation

Prepare a 5 mM Cell-ID™ Violet stock solution by adding 20 µL of anhydrous DMSO to one vial of CFSE. Vortex briefly to mix. To prepare the working solution, dilute the stock solution to final working concentration in PBS or other non-amine containing buffer just before use. Store the remaining stock solution at -80°C.

## Labeling of Cells in Suspension

1.1 Pellet cells by centrifugation and aspirate the supernatant.

1.2 Resuspend the cells in pre-warmed (37°C) PBS containing Cell-ID™ Violet at the appropriate concentration (1-10 µM).

1.3 Incubate the cells for 20 minutes at 37°C to label the cells.

1.4 Add five times the original staining volume of culture medium (containing at least 1% protein) to the cells and incubate for 5 minutes. This step removes any free dye remaining in the solution.

1.5 Pellet the labeled cells by centrifugation and resuspend in fresh pre-warmed cell culture medium.

1.6 Incubate the cells for at least 10-15 minutes at 37°C to ensure sufficient hydrolysis of Cell-ID™ Violet.

1.7 Proceed with cell stimulation, incubation, or analysis.

## Labeling of Adherent Cells

2.1 Grow cells to desired density on coverslips or chamber slides.

2.2 Remove the medium and add pre-warmed (37°C) PBS containing Cell-ID™ Violet at the appropriate concentration (1-10 µM). Use sufficient working solution to completely submerge the cells.

2.3 Incubate the cells for 20 minutes at 37°C to label the cells.

2.4 Replace the labeling solution with fresh pre-warmed cell culture medium.

2.5 Incubate for at least 10-15 minutes at 37°C to ensure sufficient hydrolysis of Cell-ID™ Violet.

2.6 Proceed with cell stimulation, incubation, or analysis.

## Fixation and Permeabilization (Optional)

3.1 Before fixation, wash and resuspend the cells in PBS or other protein-free buffer.

3.2 Fix the cells for 15–20 minutes at room temperature using an aldehyde-based fixative such as paraformaldehyde, protected from light.

3.3 Wash the cells with PBS.

3.4 If desired, permeabilize the cells using any appropriate protocol.

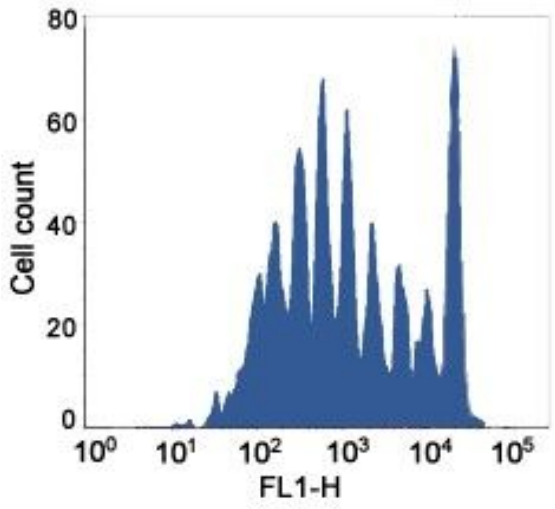
3.5 Following permeabilization, wash the cells with PBS.

3.6 Resuspend the cells in PBS prior to analysis.

## Combining with other Fluorescent Markers (Optional)

4.1 Resuspend the cells in a buffer appropriate for the subsequent staining applications.

4.2 Apply stains for immunophenotyping, DNA content, apoptosis, or other markers as recommended for each stain.




Cell generation analysis with Cell-ID™ Violet Cell Proliferation Kit. Jurkat cells (~$1\times10^6$ cells/ml) were stained with Violet dye (1 mM) on Day 0. The cells were cultured for 7 days. Fluorescence intensity was measured with FACS flow cytometer.