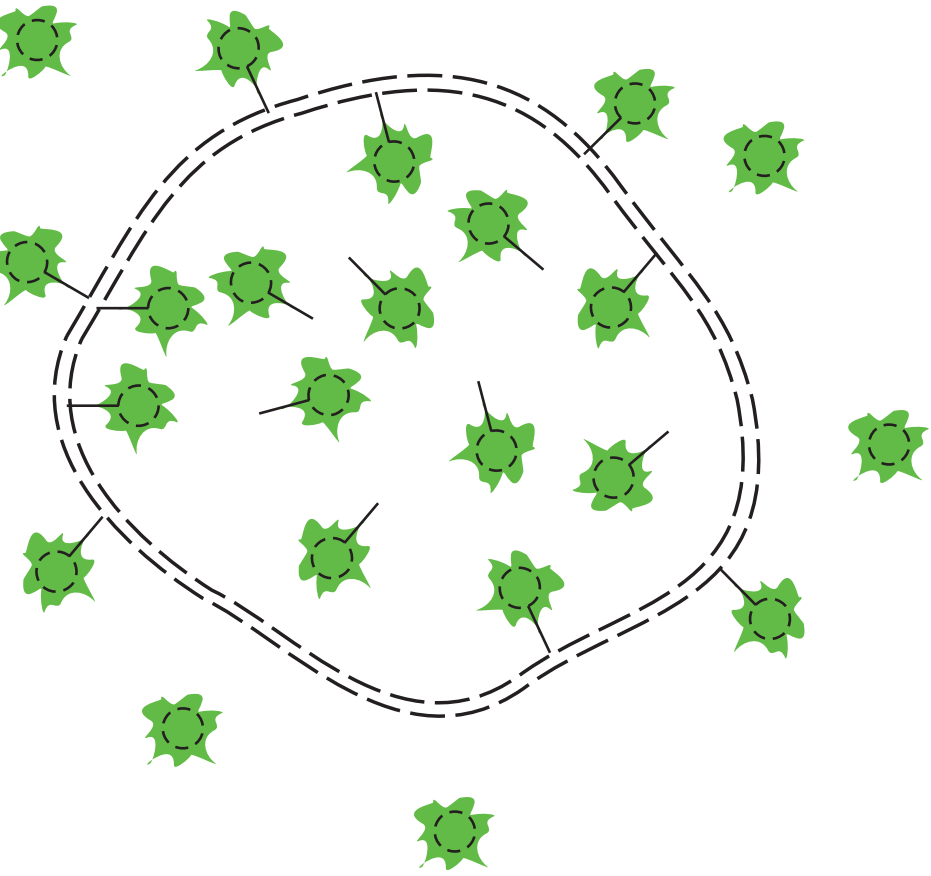

Molecular Probes® LIVE/DEAD® Fixable Dead Cell Stain Kits

Intracellular and surface staining assays for flow cytometry

Invitrogen recognizes the importance of flow cytometry in all research settings—everything from basic research to clinical diagnostics and therapeutics. Our aim is to bring together the technologies and expertise required to continue to create advances in research tools for flow cytometry.

The exciting development of the Molecular Probes® LIVE/DEAD® Fixable Dead Cell Stain Kits is one example of a novel product designed to help solve some of the challenges in your flow experiments. Find out how you now can have the flexibility to accurately distinguish live cells from dead cells after the cells have been stained and aldehyde-fixed, regardless of the application.

invitrogen™

What are the components of the LIVE/DEAD® Fixable Dead Cell Stain Kits?

This procedure is completely compatible with fixation and permeabilization methods for performing intracellular staining for flow cytometry, and with surface staining protocols as well.

→ Each kit provides sufficient materials for approximately 200 flow cytometry staining tests.

→ Kits include 5 vials of reactive dye (40 assays per vial) and one vial of DMSO.

How do LIVE/DEAD® Fixable Dead Cell Stain Kits work?

Molecular Probes® LIVE/DEAD® Fixable Dead Cell Stain Kits are based on the reaction of a fluorescent reactive dye with cellular proteins (amines).

→ For viable cells, because the dyes cannot penetrate the cell membrane, only cell surface proteins are available to react with the dye, resulting in relatively dim staining (Figure 1).

→ For dead cells, however, the reactive dye can permeate damaged membranes and stain the interior of the cell as well as the cell surface. This results in more intense staining (Figure 1).

→ The difference in fluorescence intensity between the live- and dead-cell populations is typically greater than 50-fold (Figure 2), thereby allowing complete, simultaneous discrimination of live- and dead-cell populations.

→ Because the dyes react covalently with proteins, the discrimination is completely preserved following sample fixation with formaldehyde, under conditions that inactivate pathogens.

Is staining cell line– or cell type–dependent?

No, these kits have been used successfully in our laboratories with a variety of mammalian cell types to differentially stain live and dead cells, and then fix the cells in formaldehyde for subsequent analysis by flow cytometry. Cells tested include:

→ Cell lines

- 293 MSR
- 3T3
- B35
- BPAE
- CHO-K1
- COLO205
- HeLa
- Jurkat
- K-562
- MDCK
- MRC5
- P3X
- RAW
- U205
- U266

→ Primary cells

- Human peripheral blood lymphocytes

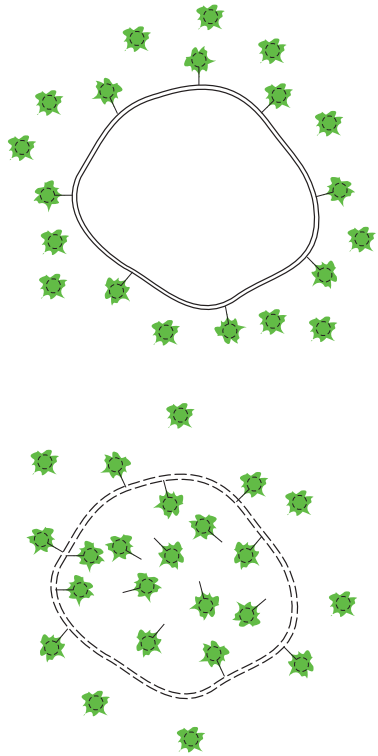

Figure 1—Principle of the LIVE/DEAD® Fixable Dead Cell Stain Kits. Live cells (top) react with the kit's fluorescent dye only on their surface to yield weak total fluorescence. Dead cells with damaged membranes (bottom) react with the dye on their surface as well as in the cell interior, yielding bright total fluorescence. In both cases, the excess reactive dye is subsequently washed away.

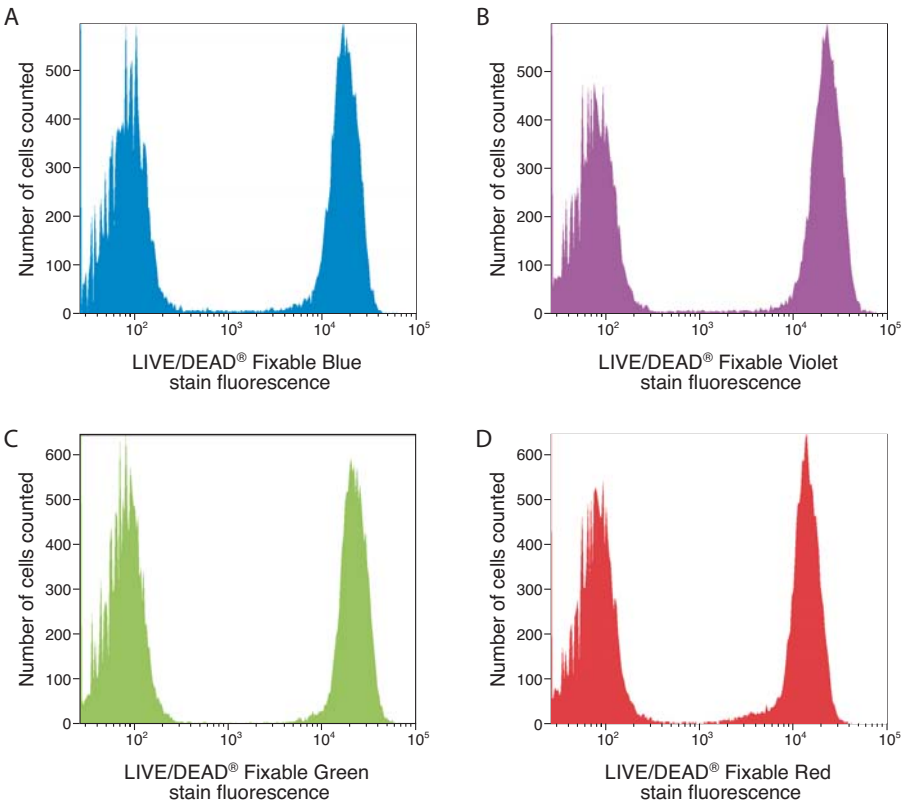

Figure 2—Live and dead cells distinguished by flow cytometry. LIVE/DEAD® Fixable Dead Cell Stain Kits were used to differentially stain a mixture of live and heat-treated Jurkat cells according to the protocol provided with the kits. Following the staining reaction, the cells were fixed in 3.7% formaldehyde and analyzed by flow cytometry. Cells were treated with **(A)** the LIVE/DEAD® Fixable Blue stain with UV excitation; **(B)** the LIVE/DEAD® Fixable Violet stain with 405 nm excitation; **(C)** the LIVE/DEAD® Fixable Green stain with 488 nm excitation; and **(D)** the LIVE/DEAD® Fixable Red stain with 488 nm excitation. The live-cell population was easily distinguished from the killed population, and nearly identical results were obtained using unfixed cells and other LIVE/DEAD® Fixable Dead Cell Stain Kits (data not shown).

Ordering information

Product	Excitation	Emission	Quantity*	Cat. no.
LIVE/DEAD® Fixable Blue Dead Cell Stain Kit	UV	450 nm	1 kit	L23105
LIVE/DEAD® Fixable Violet Dead Cell Stain Kit	405 nm	530 nm	1 kit	L34955
LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit	405 nm	525 nm	1 kit	L34957
LIVE/DEAD® Fixable Green Dead Cell Stain Kit	488 nm	440 nm	1 kit	L23101
LIVE/DEAD® Fixable Red Dead Cell Stain Kit	488 nm	630 nm	1 kit	L23102
LIVE/DEAD® Fixable Far Red Dead Cell Stain Kit	633 or 635 nm	665 nm	1 kit	L10120
LIVE/DEAD® Fixable Near-IR Dead Cell Stain Kit	633 or 635 nm	775 nm	1 kit	L10119

* Each kit allows 200 assays.

Why use LIVE/DEAD® Fixable Dead Cell Stain Kits?

Post-fixation measurement of viability

→ Nucleic acid–binding dyes like propidium iodide (PI) are not effective as dead-cell stains in these types of assays because they leach out of dead cells with washing. Also, all cells stain with PI once they are aldehyde fixed.

Accurate intracellular staining measurements

→ Induced cytokine and signaling responses are notoriously difficult to detect due to low stain signals, and this is complicated by the nonspecific binding of reagents to dead cells.

→ The stains in Molecular Probes® LIVE/DEAD® Fixable Dead Cell Stain Kits bind covalently to proteins so the discrimination of dead cells from live cells is stable, specific, and long-lived.

Reliable viability measurements

→ The "snapshot" of viability taken prior to antibody staining is maintained throughout the staining process, and beyond.

→ Dead cells often give false positive results as they tend to bind nonspecifically to many reagents. However, live- vs. dead-cell staining is unequivocal using LIVE/DEAD® Fixable Dead Cell Stain Kits.

Flexibility

→ Multiple colors for four major lasers means that these single-color assays use only one channel of one laser on a flow cytometer, leaving other channels available for more common reagents and multicolor experiments.

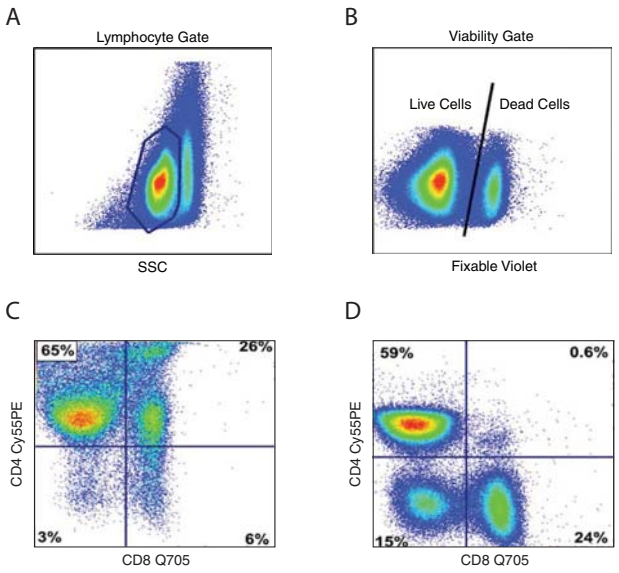

Figure 3—Exclusion of dead cells eliminates staining artifacts from analysis. In a comparison between live-cell gating using scatter **(A)** and live-cell gating using LIVE/DEAD® Fixable Violet dye **(B)**, staining artifacts using scatter gating of live cells are illustrated. The significant number of dead cells in subsequent analysis using scatter **(C)** are noted, as compared to the use of LIVE/DEAD® Fixable Violet dye **(D)** to eliminate dead cells. Reprinted from Perfetto et al. (2006), with permission from Elsevier.

Figure 4—LIVE/DEAD® Fixable Dead Cell Stain Kit. Each LIVE/DEAD® Fixable Dead Cell Stain Kit employs an amine-reactive fluorescent dye to evaluate cell viability by flow cytometry.

What is the protocol?

Dye preparation

→ Bring one vial of reactive dye (Component A) and the vial of anhydrous DMSO (Component B) to room temperature before removing the caps.

→ Add 50 µl of DMSO to the vial of dye and mix well.

→ Use the solution of reactive dye, ideally within a few hours of reconstitution.

*Cell staining**

→ Centrifuge a cell suspension containing at least 1 x 10^6 cells. Discard the supernatant.

→ Wash cells with PBS and resuspend in 1.0 ml of PBS.

→ Count cells and adjust density with PBS to 1 x 10^6 cells/ml.

→ Add 1 µl of reconstituted dye to 1 ml of the cell suspension and mix well.

→ Incubate at room temperature or on ice for 30 minutes.

→ Wash with PBS and resuspend in 100 µl of cell staining buffer.

→ Stain as usual using extracellular or intracellular antibodies or other reagents. Fix with an aldehyde-based fixative prior to permeabilization. Samples may be analyzed without fixation and permeabilization, if desired.

→ Analyze in a flow cytometer.

* Buffers appropriate for cell staining include phosphate-buffered saline (PBS), Hanks' Balanced Salt Solution (HBSS), and Dulbecco's PBS, without extraneous proteins such as bovine serum albumin or serum. With these kits, do not use Tris buffers or solutions containing sodium azide or extraneous proteins for cell resuspension or washing.

How do I use these kits with my existing intracellular (IC) staining protocol?

If another staining reaction is to be performed on the same sample, the user must determine the optimal staining sequence for the two procedures and whether or not the additional staining reaction will tolerate fixation by formaldehyde.

Do the dyes interfere with IC antibody conjugate fluorescence?

Although this is a commonly asked question, to date there have been no reports that the binding of these dyes interferes with surface antibody binding.

What is the shelf life of the kits?

Kits are stable for 6 months when stored at –20°C, desiccated and protected from light. Once a single vial of dye is reconstituted with the DMSO solution, the reactive dye is stable for up to 2 weeks if stored at –20°C or lower, protected from light and moisture.

Are there publications citing these kits?

Yes. Here are some selected recent publications.

Multiple dyes, such as amine-reactive viability dyes (ViD), green fluorescence (GrViD), orange fluorescence (OrViD), violet fluorescence (ViViD), UV fluorescence (UViD)

Perfetto, S.P. et al. (2006) Amine reactive dyes: an effective tool to discriminate live and dead cells in polychromatic flow cytometry. *J Immunol Methods* 313:199–208.

LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit

Burmeister, Y. et al. (2008) ICOS controls the pool size of effector-memory and regulatory T cells. *J Immunol* 180:774–782.

Badr, G. et al. (2008) Early interferon therapy for HCV rescues poly-functional long-lived $CD8^+$ memory T cells. *J Virol* doi:10.1128/JVI.01083–08.

LIVE/DEAD® Fixable Violet Dead Cell Stain Kit

Singh, P. et al. (2008) Vaccinia virus infection modulates the hematopoietic cell compartments in the bone marrow. *Stem Cells* 26:1009–1016.

Charles, E.D. et al. (2008) Clonal expansion of immunoglobulin M^+CD27^+ B cells in HCV-associated mixed cryoglobulinemia. *Blood* 111:1344–1356.

LIVE/DEAD® Fixable Blue Dead Cell Stain Kit

Woodland, R.T. et al. (2008) Multiple signaling pathways promote B lymphocyte stimulator dependent B-cell growth and survival. *Blood* 111:750–760.

LIVE/DEAD® Fixable Red Dead Cell Stain Kit

Alici, E. et al. (2008) Autologous antitumor activity by NK cells expanded from myeloma patients using GMP-compliant components. *Blood* 111:3155–3162.

Serkova, N.J. et al. (2008) Utility of magnetic resonance imaging and nuclear magnetic resonance-based metabolomics for quantification of inflammatory lung injury. *Am J Physiol Lung Cell Mol Physiol* 295:L152–L161.

Figure 3 reprinted from Perfetto et al. (2006), with permission from Elsevier.