LIVE/DEAD® Violet Viability/Vitality Kit

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
CellTrace™ calcein violet stain, lyophilized (Component A) *	5 vials, 25 µg each	• ≤–20°C • Desiccate • Protect from light	When stored as directed, kit components should remain stable for at least 6 months.
Aqua-fluorescent reactive dye, lyophilized (Component B)	5 vials		
DMSO, anhydrous (Component C)	500 µL		

* Calcein violet AM may hydrolyze if exposed to moisture.

Number of labelings: At the recommended reagent concentrations and volumes, this kit contains sufficient material to perform approximately 200 assays using flow cytometry.

Approximate fluorescence excitation/emission maxima: calcein violet 400/452 nm; aqua-fluorescent reactive dye 367/526 nm

Introduction

The LIVE/DEAD® Violet Viability/Vitality Kit provides a two-color fluorescence cell viability and vitality assay that is based on the simultaneous determination of live and dead cells with two probes that measure recognized parameters of cell health: plasma membrane integrity as a measure of cell viability, and intracellular esterase activity as a measure of cell vitality. Calcein violet AM and aqua-fluorescent reactive dye are optimal dyes for this application; both dyes utilize the violet laser allowing other laser lines to be used with more conventional markers.

Live cells are distinguished from dead cells by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually non-fluorescent cell-permeant calcein violet AM to the intensely fluorescent calcein violet. In contrast, dead cells are deficient in esterase activity and do not convert calcein AM to its fluorescent form. However, dead cells can be positively distinguished from live cells using the aqua-fluorescent amine-reactive dye. The reactive dye can permeate the compromised membranes of dead cells and react with free amines in the interior of the cell, resulting in intense fluorescent staining of dead cells (Figures 1 and 2). In contrast, live cells exclude the amine-reactive dye and do not exhibit strong fluorescence.

Principle of the method

The acetoxymethyl (AM) ester derivatives of fluorescent indicators make up one of the most useful groups of compounds for the study of live cells. Modification of carboxylic acids with AM ester groups results in an uncharged molecule that can permeate cell membranes. Once inside the cell, the lipophilic blocking groups are cleaved by nonspecific esterases, resulting in a charged form that is retained in cells to a much greater extent than its parent compound. The calcein violet AM ester is colorless and non fluorescent until hydrolyzed. The polyanionic dye calcein violet is well retained within live cells, producing an intense uniform violet fluorescence in live cells (ex/em 400/452 nm).

Based on the reaction of a dye that is reactive with cellular amines, dead cells may be positively distinguished from living cells. The aqua-fluorescent reactive dye can permeate the compromised membranes of dead cells and react with free amines both in the interior and on the cell surface, resulting in intense fluorescent staining (ex/em 367/526 nm). In contrast, only the cell-surface amines of viable cells are available to react with the dye, resulting in relatively dim staining.

The determinations of cell viability and cell vitality depends on these physical and biochemical properties of the cells. As cells die, variations in fluorescence will be observed.

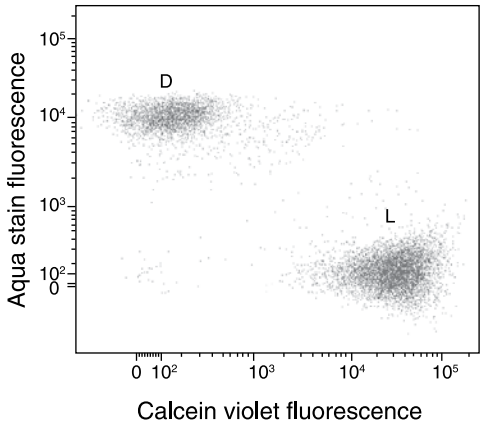

Figure 1. Staining pattern of a mixture of heat-killed and untreated Jurkat cells (human leukemia T-cell). Jurkat cells were stained according to the protocol in the LIVE/DEAD® Violet Viability/Vitality Kit. Cells were analyzed using a flow cytometer equipped with a 405 nm laser and a 450/50 bandpass filter for calcein violet–labeled live cells (L) and 525/50 bandpass for the aqua-fluorescent reactive dye–labeled dead cells (D).

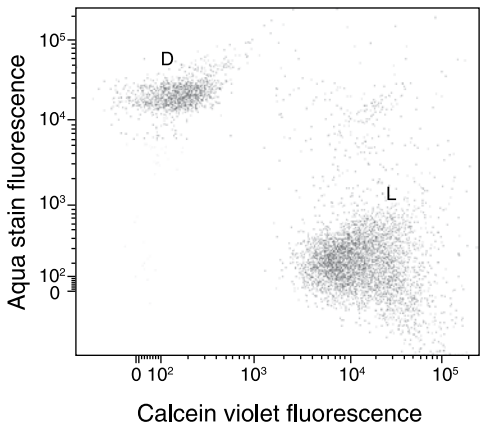

Figure 2. Staining pattern of a mixture of heat-killed and untreated Chinese hamster ovary cells (CHO cells). CHO cells were harvested using TyrpLE™ Express (Invitrogen Cat. no. 12605-010) and stained according to the protocol in the LIVE/DEAD® Violet Viability/Vitality Kit. Cells were analyzed using a flow cytometer equipped with a 405 nm laser and 450/50 bandpass for calcein violet–labeled live cells (L) and 525/50 bandpass for the aqua-fluorescent reactive dye–labeled dead cells (D).

Before You Begin

Allow the components to warm to room temperature before opening the vials.

Working with the Stock Solutions

Calcein violet AM is susceptible to hydrolysis when exposed to moisture. Once prepared, DMSO stock solutions of calcein violet AM should preferably be used within a short time period for one set of experiment, while aqueous working solutions containing calcein violet AM should be prepared immediately prior to use and used within one day. Once the aqua-fluorescent reactive dye is reconstituted with DMSO, it is somewhat unstable if exposed to moisture.

Caution

Hazards posed by these stains have not been fully investigated. DMSO is known to facilitate entry of organic molecules into tissue. These reagents should be handled using equipment and practices appropriate for the hazards posed by such material. Please dispose of reagents in compliance with all pertaining local regulations.

Experimental Protocol

This flow cytometry protocol has been optimized using Jurkat cells (human T-cell leukemia line) at a concentration of 1×10^6 cells/mL. Use of other cell types or other cell concentrations may require optimization of staining. If another staining reaction is to be performed on the sample, the user must determine the optimal staining sequence for the two procedures.

1.1 Allow one vial of each Component A , Component B, and Component C to come to room temperature.

1.2 Prepare a 1 mL suspension of cells with $0.1–5 \times 10^6$ cells/mL for each assay. Cells may be suspended in medium or buffer.

1.3 Add 50 µL DMSO (Component C) to one vial aqua-fluorescent reactive dye (Component B).

1.4 Add 42 µL DMSO (Component C) to one vial calcein violet AM (Component A) to prepare a stock solution. Add 40 µL of this stock solution to 1.25 mL of buffer or medium to make a working solution of calcein violet AM. This working solution should be used within one day.

1.5 Add 1 µL aqua-fluorescent reactive dye working solution and 5 µL calcein violet AM working solution to each mL cell suspension. Mix the sample.

1.6 Incubate the cells for 30 minutes at room temperature or on ice.

1.7 Wash once and resuspend in buffer.

1.8 Analyze the stained cells by flow cytometry using violet (~405 nm) excitation and violet fluorescence emission (~450 nm) for the calcein violet (live cells) and blue-green fluorescence emission (~525 nm) for the aqua-fluorescent reactive dye (dead cells). Minimal compensation will be necessary.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
L34958	LIVE/DEAD® Violet Laser Viability/Cytotoxicity Kit	1 kit

Contact Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:
6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:
8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License No. 223: Labeling and Detection Technology
The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.