LIVE/DEAD® Viability/Cytotoxicity Kit *for mammalian cells*

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Protect from light

Ex/Em

- Calcein = 494/517 nm
- Ethidium homodimer-1 in the presence of DNA = 528/617 nm

Note: Calcein AM may hydrolyze if exposed to moisture.

Introduction

The LIVE/DEAD® Viability/Cytotoxicity Assay Kit provides a two-color fluorescence cell viability assay that is based on the simultaneous determination of live and dead cells with two probes that measure recognized parameters of cell viability—intracellular esterase activity and plasma membrane integrity. Molecular Probes has found that calcein AM and ethidium homodimer (EthD-1) are optimal dyes for this application.[1-3] The kit is suitable for use with fluorescence microscopes or fluorescence multiwell plate scanners and easily adaptable for use with flow cytometers and other fluorescence detection systems. The assay principles are general and applicable to most eukaryotic cell types, including adherent cells [4] and certain tissues,[5,6] but not to bacteria or yeast.[3] This fluorescence-based method of assessing cell viability can be used in place of trypan blue exclusion, ^{51}Cr release and similar methods for determining cell viability and cytotoxicity. It is generally faster, less expensive, safer and a more sensitive indicator of cytotoxic events than alternative methods. Validity of the LIVE/DEAD® Viability/Cytotoxicity assay for animal cell applications has been established by several laboratories. Published applications include measuring the cytotoxic effects of tumor necrosis factor (TNF),[7] β-amyloid protein,[8] adenovirus E1A proteins,[9] tetrodotoxin (TTX) binding to Na^+ channels,[10] methamphetamines [11] and mitogenic sphingolipids.[12] The assay has also been utilized to quantitate apoptotic cell death [13,14] and cell-mediated cytotoxicity.[15,16]

Principle of the Method

Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually nonfluorescent cell-permeant calcein AM to the intensely fluorescent calcein. The polyanionic dye calcein is well retained within live cells, producing an intense uniform green fluorescence in live cells (ex/em ~495 nm/~515 nm). EthD-1 enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells (ex/em ~495 nm/~635 nm). EthD-1 is excluded by the intact plasma membrane of live cells. The determination of cell viability depends on these physical and biochemical properties of cells. Cytotoxic events that do not affect these cell properties may not be accurately assessed using this method. Background fluorescence levels are inherently low with this assay technique because the dyes are virtually non-fluorescent before interacting with cells.

Kit Contents

- **Calcein AM** (Component A), two vials, 40 µL each, 4 mM in anhydrous DMSO
- **Ethidium homodimer-1** (Component B), two vials, 200 µL each, 2 mM in DMSO/H_2O 1:4 (v/v)

At the recommended reagent concentrations and volumes, this kit contains sufficient material to perform approximately 1,000 tests using a fluorescence microscope or fluorescence microplate reader or approximately 100 tests using a flow cytometer.

Storage and Handling of Reagents

Reagents in this kit should be stored sealed, desiccated, protected from light and frozen at ≤–20˚C. Allow the reagents to warm to room temperature and centrifuge briefly before opening. Before refreezing, seal all stock solutions tightly. Calcein AM is susceptible to hydrolysis when exposed to moisture. Prepare aqueous working solutions containing calcein AM immediately prior to use, and use within one day. EthD-1 is stable and insensitive to moisture. Stock solutions of EthD-1 in DMSO/ H_2O or other aqueous media can be stored frozen at ≤–20°C for at least one year.

Fluorescence Microscopy Protocol

Select the Optical Filters

Calcein and EthD-1 can be viewed simultaneously with a conventional fluorescein longpass filter. The fluorescence from these dyes may also be observed separately; calcein can be viewed with a standard fluorescein bandpass filter and EthD-1 can be viewed with filters for propidium iodide or Texas Red® dye. Typical characteristics of some appropriate filters are summarized in Table 1.

Prepare the Cells

1.1 Adherent cells may be cultured on sterile glass coverslips as either confluent or subconfluent monolayers (e.g., fibroblasts are typically grown on the coverslip for 2–3 days until acceptable cell densities are obtained). The cells may be cultured inside 35 mm disposable petri dishes or other suitable containers; non-adherent cells may also be used.

1.2 Wash the cells prior to the assay to remove or dilute serum esterase activity generally present in serum-supplemented growth media (serum esterases could cause some increase in extracellular fluorescence by hydrolyzing calcein AM). Wash adherent cells gently with 500–1,000 volumes of Dulbecco's phosphate-buffered saline (D-PBS) (note A).

1.3 Wash non-adherent cells in a test tube with 500–1,000 volumes of tissue culture–grade D-PBS and sediment by centrifugation. Transfer an aliquot of the cell suspension to a coverslip. Allow cells to settle to the surface of the glass coverslip at 37°C in a covered 35 mm petri dish.

1.4 Treat the cells with cytotoxic agents as required at any time prior to or concurrent with LIVE/DEAD® reagent staining.

Determine the Optimal Dye Concentrations

Best results are obtained by adjusting the dye concentrations to achieve distinct labeling of live cells with calcein AM and of dead cells with EthD-1. The optimal concentrations are likely to vary depending on the cell type. In general it is best to use the lowest dye concentration that gives sufficient signal. The following method can be used to determine optimal dye concentrations:

2.1 Remove the LIVE/DEAD® assay reagents from the freezer and allow them to warm to room temperature.

Table 1. Characteristics of common filters suitable for use with the LIVE/DEAD® Viability Kit

Omega Filters*	Chroma Filters*	Notes
XF25, XF26, XF115	11001, 41012, 71010	Longpass and dual emission filters useful for simultaneous viewing of calcein and EthD-1 stains
XF22, XF23	31001, 41001	Bandpass filters for viewing calcein alone
XF32, XF43, XF102, XF108	31002, 31004, 41002, 41004	Bandpass filters for viewing EthD-1 alone

* Catalog numbers for recommended bandpass filter sets for fluorescence microscopy. Omega filters are supplied by Omega Optical Inc. (www.omegafilters.com). Chroma filters are supplied by Chroma Technology Corp. (www.chroma.com).

2.2 Prepare some samples of live cells as well as of dead cells on glass coverslips. Kill the cells using any preferred method (e.g., treatment with 0.1% saponin for 10 minutes, 0.1–0.5% digitonin for 10 minutes, 70% methanol for 30 minutes or complement and the appropriate IgG for 30 minutes).

2.3 Using samples of dead cells, select an EthD-1 concentration that stains the dead cell nuclei bright red without staining the cytoplasm significantly (try from 0.1 to 10 µM EthD-1).

2.4 Using samples of dead cells, select a calcein AM concentration that does not give significant fluorescence in the dead cell cytoplasm (try from 0.1 to 10 µM calcein AM).

2.5 Using samples of live cells, check to see that the calcein AM concentration selected in step 2.4 generates sufficient fluorescence signal in live cells (if not, try a higher concentration).

2.6 The reagent concentrations determined in steps 2.3 and 2.5 are optimal for the viability experiments.

Example Dilution Protocol

This example protocol makes 10 mL of an approximately 2 µM calcein AM and 4 µM EthD-1 solution. We found these dye concentrations to be suitable for NIH 3T3, PtK2 and MDCK cells when incubated at room temperature for 20–40 minutes. Cultured mouse leukocytes (J774A.1), which have higher esterase activity, require 5–10 times less calcein AM than that required for the three other cell types, but the same amount of EthD-1. This is an example protocol only; the optimal dye concentrations for any experiment will vary.

3.1 Remove theLIVE/DEAD® reagent stock solutions from the freezer and allow them to warm to room temperature.

3.2 Add 20 µL of the supplied 2 mM EthD-1 stock solution (Component B) to 10 mL of sterile, tissue culture–grade D-PBS, vortexing to ensure thorough mixing. This gives an approximately 4 µM EthD-1 solution.

3.3 Combine the reagents by transferring 5 µL of the supplied 4 mM calcein AM stock solution (Component A) to the 10 mL EthD-1 solution. Vortex the resulting solution to ensure thorough mixing.

3.4 The resulting approximately 2 µM calcein AM and 4 µM EthD-1 working solution is then added directly to cells. The final concentration of DMSO is ≤ 0.1%, a level generally innocuous to most cells.

3.5 Note that aqueous solutions of calcein AM are susceptible to hydrolysis (see Storage and Handling of Reagents). Aqueous working solutions should therefore be used within one day.

Perform the Viability Assay

4.1 Add 100–150 µL of the combined LIVE/DEAD® assay reagents, using optimized concentrations, to the surface of a 22 mm square coverslip, so that all cells are covered with solution. Incubations should be performed in a covered dish (e.g., 35 mm disposable petri dish) to prevent contamination or drying of the samples.

4.2 Incubate the cells for 30–45 minutes at room temperature. A shorter incubation time may be used if the dye concentrations or incubation temperature are increased.

4.3 Following incubation, add about 10 µL of the fresh LIVE/DEAD® reagent solution or D-PBS to a clean microscope slide.

4.4 Using fine-tipped forceps, carefully (but quickly) invert and mount the wet coverslip on the microscope slide. To prevent evaporation, seal the coverslip to the glass slide (e.g., with clear fingernail polish). Avoid damaging or shearing cells in the preparation of the slides.

4.5 View the labeled cells under the fluorescence microscope.

Fluorescence Microplate Protocol

Select the Optical Filters for the Microplate Reader

In order to obtain the greatest sensitivity using a plate reader, we recommend exciting the fluorophores using optical filters optimal for their respective absorbances. Calcein is well excited using a fluorescein optical filter (485 ± 10 nm) whereas EthD-1 is compatible with a typical rhodamine optical filter (530 ± 12.5 nm). The fluorescence emissions should be acquired separately as well, calcein at 530 ± 12.5 nm, and EthD-1 at 645 ± 20 nm.

Prepare the Cells for the Microplate Reader

5.1 Culture adherent cells in the multiwell plate. Fibroblast cells are typically grown in the wells for 2–3 days until acceptable cell densities are obtained. Wash the cells gently with 500–1000 volumes of Dulbecco's phosphate-buffered saline (D-PBS) prior to the assay (note A). After the last wash, add sufficient D-PBS to at least cover the bottom of the well. The cell samples are washed to remove or to dilute esterase activity generally present in serum-supplemented growth media that could cause an increase in extracellular fluorescence due to hydrolysis of calcein AM.

5.2 Wash relatively nonadherent cells (e.g., leukocytes or other suspended cells) in a test tube with 500–1000 volumes of tissue culture–grade D-PBS and sediment by centrifugation to remove serum esterase activity.

5.3 Add the cells in a sufficient volume of buffer to at least cover the bottom of the wells. In general, for flat-bottomed wells where the total capacity is 250–300 µL, add about 100 µL; for round-bottomed wells where the total capacity is 150–200 µL, add about 70 µL; for conical wells where the total capacity is 100–150 µL, add about 50 µL. Small buffer volumes may be preferred to minimize dilution of cytotoxic agents and other reagents.

5.4 Treat the cells with cytotoxic agents as required at any time prior to or concurrent with LIVE/DEAD® reagent staining.

5.5 The minimum detectable number of cells per well is usually between 200 and 500. The maximum usable number of cells per well is on the order of 10^6.

Determine the Optimal Dye Concentrations

Best results are obtained by adjusting the dye concentrations to achieve distinct labeling of live cells with calcein AM and dead cells with EthD-1. Changes in optical filters, instrument sensitivity settings and numbers or types of cells may require different dye concentrations. In general, it is best to use the lowest dye concentration that gives sufficient signal. The following method can be used to determine optimal dye concentrations:

6.1 Remove the LIVE/DEAD® reagents from the freezer and allow them to warm to room temperature. Select appropriate filters and settings on the plate reader.

6.2 Prepare samples of live cells as well as of dead cells. Kill the cells using any preferred method (e.g., treatment with 0.1% saponin for about 10 minutes, 0.1–0.5% digitonin for about 10 minutes, 70% methanol for about 30 minutes or complement and the appropriate IgG for about 30 minutes).

6.3 Using samples of dead cells, determine the saturating concentration of EthD-1 (the lowest concentration that still yields maximal fluorescence). Try from 0.1 to 10 µM of EthD-1, while maintaining a constant high cell concentration (about 10^6 cells per mL). Monitor the time course of staining to determine optimum incubation times (try taking measurements every 10–15 minutes). We found a 45 minute incubation in 4 µM EthD-1 saturates the binding sites in a sample of 120,000 killed mouse leukocytes.

6.4 Using samples of dead cells, determine concentrations of calcein AM that give negligible staining of dead cells (try from 0.1 to 5 µM calcein AM).

6.5 Using samples of live cells, determine the concentration of calcein AM that gives fluorescence in live cells sufficient to permit clear detection. If the signal is too low, increase the number of cells or use a slightly higher concentration of the dye.

6.6 The reagent concentrations determined in steps 6.3 and 6.5 are optimal for the viability assay.

Sample Preparation Example for Microplate Reader Measurements

This example protocol makes 10 mL of the LIVE/DEAD® reagents for use in a multiwell plate scanner at 1 µM calcein AM and 2 µM EthD-1 (we found these reagent concentrations to be optimal for mouse leukocytes). The protocol prepares a 2X concentrated reagent stock to allow for a final two-fold dilution upon addition to the wells. Ten milliliters of the stock solution at 100 µL per test gives enough dye solution for one 96-well microplate. This is an example protocol only; the actual volumes and concentrations used in an experiment will depend on the type of cells and microplates used.

7.1 Remove the LIVE/DEAD® reagent stock solutions from freezer and allow them to warm to room temperature.

7.2 Add 20 µL of the supplied 2 mM EthD-1 stock solution (Component B) to 10 mL of sterile, tissue culture–grade D-PBS, vortexing to ensure thorough mixing. This gives an approximately 4 µM EthD-1 solution.

7.3 Transfer a 5 µL aliquot of the supplied 4 mM calcein AM solution in DMSO (Component A) to the 10 mL of 4 µM EthD-1 solution. Vortex or sonicate the resulting solution to ensure

thorough mixing. This gives an approximately 2 µM calcein AM and 4 µM EthD-1 working solution.

7.4 Distribute 100 µL of cell-containing buffer to each well. Add an additional 100 µL of the LIVE/DEAD® working solution, yielding 200 µL per well containing 1 µM calcein AM and 2 µM EthD-1. The final concentration of DMSO is ≤ 0.1%, a level generally innocuous to most cells.

Fluorescence Measurements Using a Microplate Reader

8.1 Prepare the samples of experimental cells (A and B below) and of live and dead cell controls (C through F below).

8.2 The set of control measurements is included to account for sources of background fluorescence, which can then be factored out in subsequent calculations. Treat the experimental and control cell samples identically (i.e., maintain constant cell numbers, reagent concentrations, and incubation times and temperatures). Label the experimental cells with calcein AM and EthD-1. Label the control samples as indicated with either calcein AM or EthD-1. A cell-free control (G and H below) may be included to test for background fluorescence from the cytotoxic agent being tested or from other additives in the medium.

8.3 Add the LIVE/DEAD® reagents to the wells to the optimal final concentrations (described in *Determine the Optimal Dye Concentrations*).

8.4 Incubate the samples for the optimal time interval (described in *Determine the Optimal Dye Concentrations*), e.g., at room temperature for 30–45 minutes.

8.5 Measure the fluorescence in the experimental and control cell samples using the appropriate excitation and emission filters:

A. Fluorescence at 645 nm in the experimental cell sample, labeled with calcein AM and EthD-1 = $\mathbf{F(645)_{sam}}$
B. Fluorescence at 530 nm in the experimental cell sample, labeled with calcein AM and EthD-1 = $\mathbf{F(530)_{sam}}$
C. Fluorescence at 645 nm in a sample where all the cells are dead, labeled with EthD-1 only = $\mathbf{F(645)_{max}}$
D. Fluorescence at 645 nm in a sample where all the cells are dead, labeled with calcein AM only = $\mathbf{F(645)_{min}}$
E. Fluorescence at 530 nm in a sample where all (or nearly all) cells are alive, labeled with EthD-1 only = $\mathbf{F(530)_{min}}$
F. Fluorescence at 530 nm in a sample where all (or nearly all) cells are alive, labeled with calcein AM only = $\mathbf{F(530)_{max}}$
G. Fluorescence at 530 nm of the cell-free sample with or without dye added = $\mathbf{F(530)_0}$
H. Fluorescence at 645 nm of a cell-free sample with or without dye added = $\mathbf{F(645)_0}$

Interpretation of the Results

The relative numbers of live and dead cells can be expressed in terms of percentages or as absolute numbers of cells (described in *Determining Absolute Numbers of Live and Dead Cells*) at about 530 nm and limited fluorescence signal at longer wavelengths. Dead cells are characterized by intense fluorescence at >600 nm and little fluorescence around 530 nm. Background fluorescence readings ($F(530)_0$ and $F(645)_0$) may be subtracted from all values of F(530) and F(645) respectively prior to calculation of results.

The percentage of live cells can be calculated from the fluorescence readings defined above as:

$$\%\ \text{Live Cells} = \frac{F(530)_{sam} - F(530)_{min}}{F(530)_{max} - F(530)_{min}} \times 100\%$$

The percentage of dead cells can be calculated from the fluorescence readings defined above as:

$$\%\ \text{Dead Cells} = \frac{F(645)_{sam} - F(645)_{min}}{F(645)_{max} - F(645)_{min}} \times 100\%$$

Determining Absolute Numbers of Live and Dead Cells Using a Microplate Reader

The total number of cells in a sample can be counted by killing all of the cells (see step 6.2), labeling with a saturating concentration of EthD-1 and measuring fluorescence at >600 nm. The fluorescence intensity is then linearly related to the total number of cells present in the sample. This may be done at the end of a set of viability experiments in order to express cell viability in terms of absolute numbers of live and dead cells.

9.1 Perform the cell-viability measurements (described in *Fluorescence Measurements Using Microplate Reader*).

9.2 Kill all of the cells in the samples (e.g., by adding about 0.1% saponin to each well; add 2–5 µL per well from a 5% saponin stock solution in distilled water).

9.3 Mix by shaking the plate; wait 10 minutes (or until the signal equilibrates).

9.4 Read the EthD-1 fluorescence at ~645 nm. The fluorescence intensity is linearly related to the number of cells in the sample. This value can be compared to a standard curve of numbers of dead cells vs fluorescence intensity, generated separately by using a saturating EthD-1 concentration on known numbers of dead cells in a microplate.

Flow Cytometry Protocol: Viability Assay

10.1 Allow all reagents to come to room temperature.

10.2 Make an 80-fold dilution of calcein AM (Component A) in DMSO to make a 50 µM working solution (i.e., add 2 µL of Component A to 158 µL DMSO). The working solution should be used within one day.

10.3 Prepare a 1 mL suspension of cells with 0.1 to 5×10^6 cells/mL for each assay. Cells may be in culture medium or buffer.

10.4 Add 2 µL of 50 µM calcein AM working solution and 4 µL of the 2 mM ethidium homodimer-1 stock to each milliliter of cells. Mix the sample.

10.5 Incubate the cells for 15–20 minutes at room temperature, protected from light.

10.6 As soon as possible after the incubation period (within 1–2 hours), analyze the stained cells by flow cytometry using 488 nm excitation and measuring green fluorescence emission for calcein (i.e., 530/30 bandpass) and red fluorescence emission for ethidium homodimer-1 (i.e., 610/20 bandpass). Gate on cells to exclude debris. Using single color stained cells, perform standard compensation. The population should separate into two groups: live cells will show green fluorescence and dead cells will show red fluorescence (Figure 1).

Flow Cytometry Protocol: Viability Assay with CountBright™ Absolute Counting Beads

Note: The accuracy of cell counts based on CountBright™ absolute counting beads depends on sample handling and the precise delivery of the volume of beads. The CountBright™ absolute counting beads must be mixed well to assure a uniform suspension of microspheres; vortex for 30 seconds immediately before removing an aliquot. Cell suspensions may be diluted, but should be assayed without wash steps.

11.1 Allow all reagents to thaw completely.

11.2 Make an 80-fold dilution of calcein AM (Component A) in DMSO to make a 50 µM working solution (i.e., add 2 µL of Component A to 158 µL DMSO). The working solution should be used within one day.

11.3 Prepare a 1 mL suspension of cells with 0.1 to 5×10^6 cells/mL for each assay. Cells may be in culture medium or buffer.

11.4 Add 2 µL of 50 µM calcein AM solution and 4 µL of the 2 mM ethidium homodimer-1 stock per mL of cells. Mix the sample.

11.5 Incubate the cells for 15–20 minutes at room temperature, protected from light.

11.6 Allow the CountBright™ absolute counting beads to come to room temperature. Gently vortex the microsphere suspension for 30 seconds to completely resuspend.

11.7 Immediately after vortexing the counting bead suspension, add 50 µL of counting beads to each milliliter of sample and vortex.

Note: At this dilution, the small amount of Tween 20 and sodium azide contributed by the CountBright™ absolute counting beads has not been noted to affect cell staining or viability.

11.8 As soon as possible after the incubation period, analyze the sample by flow cytometry using 488 nm excitation and measuring green fluorescence emission for calcein (i.e., 530/30 bandpass) and red fluorescence emission for ethidium homodimer-1 (i.e., 610/20 bandpass). Gate both on cells (to exclude debris) and on counting beads. Set forward scatter threshold low enough to include the microspheres on the forward vs side scatter plot (Figure 2). Using single color stained cells, perform standard compensation. The population should separate into two groups: live cells will show green fluorescence and dead cells will show red fluorescence. The CountBright™ absolute counting beads can be distinguished from cells (Figure 3).

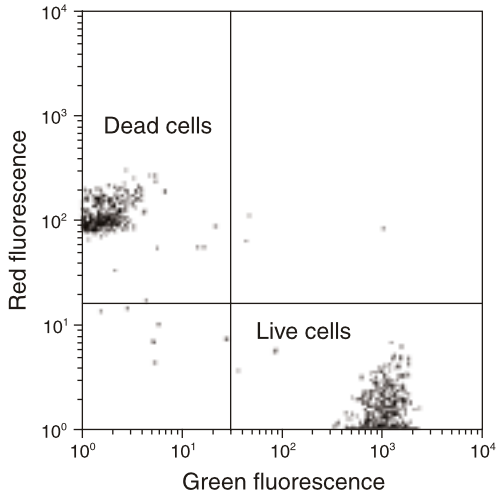

Figure 1. Flow cytometric viability assay using our LIVE/DEAD® Viability/Cytotoxicity Kit. A 1:1 mixture of live and ethanol-fixed human B cells was stained with calcein AM and ethidium homodimer-1 following the protocol provided. Flow cytometry analysis was carried out with excitation at 488 nm. The resulting bivariate frequency distribution shows the clear separation of the green-fluorescent (530 nm) live-cell population from the red-fluorescent (585 nm) dead-cell population.

Note: Collect at least 1,000 bead events to assure a statistically significant determination of sample volume.

11.9 The counting beads should appear in the upper right corner of all fluorescence plots (Figure 3), and can be gated accordingly.

Note: If the CountBright™ absolute counting beads cannot be resolved from cells in a particular emission parameter combination, use a different combination of emission parameters to gate the counting beads.

Calculation of cell concentration:

$$\frac{A}{B} \times \frac{C}{D} = \text{concentration of sample as cells/µL}$$

Where:
A = number of cell events
B = number of bead events
C = assigned bead count of the lot (beads/50 µL)
D = volume of sample (µL)

Example calculation: A 1,000 µL volume of cells was stained. Afterwards, 50 µL of CountBright™ absolute counting beads was added.

$$\frac{1{,}700 \text{ cells}}{1{,}030 \text{ beads}} \times \frac{49{,}500 \text{ beads}/50 \text{ µL}}{1{,}000 \text{ µL}} = 81.7 \text{ cells/µL}$$

Note: The calculation should be corrected if the sample is diluted or if a different volume of CountBright™ absolute counting beads is used.

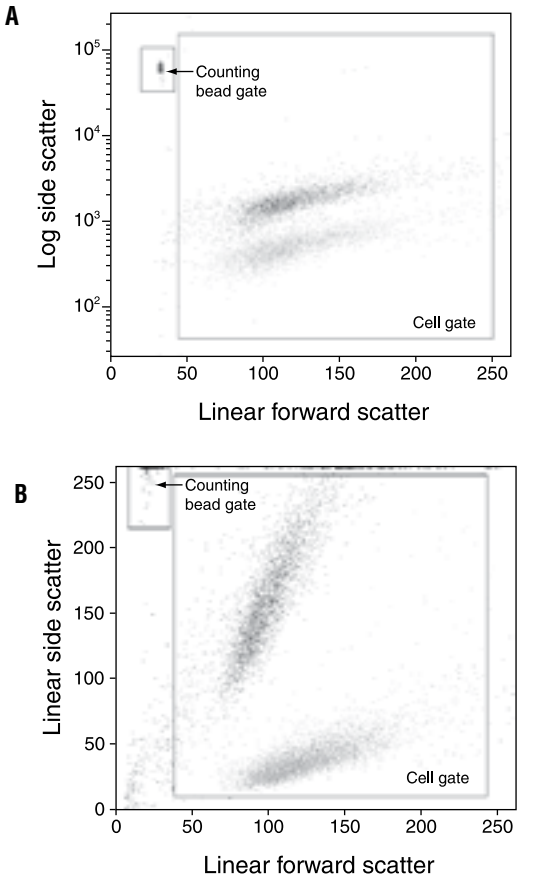

Figure 2. Counting bead gating on forward vs side scatter. A mixture of live and heat-killed Jurkat cells (human T-cell leukemia) was stained with calcein AM and ethidium homodimer-1 following the protocol provided. CountBright™ absolute counting beads were added prior to data acquisition on the flow cytometer. A) Forward scatter vs logarithmic side scatter shows gating of cells to exclude debris as well as gating of counting beads. B) Forward scatter vs linear side scatter shows gating of cells to exclude debris with gating of counting beads. The counting bead gate is adjusted to include the last channel in side scatter.

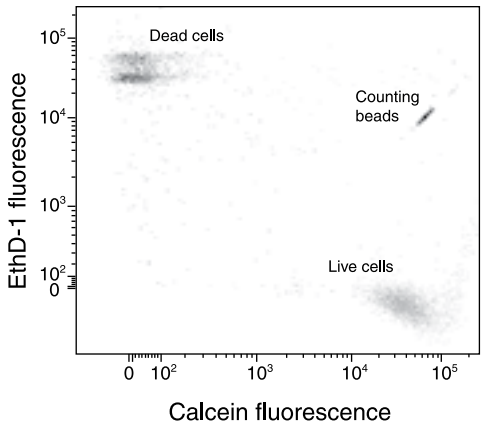

Figure 3. Plot of calcein fluorescence collected through a 530/30 bandpass filter vs ethidium homodimer-1 fluorescence collected through a 610/20 bandpass filter, showing clear separation of live and dead cells, as well as counting beads. A mixture of live and heat-killed Jurkat cells was stained with calcein AM and ethidium homodimer-1 following the protocol provided. CountBright™ absolute counting beads were added prior to data acquisition on the flow cytometer using 488 nm excitation.

Note

[A] Any standard saline buffer may be used throughout these protocols. Colored additives like phenol red should be checked, however, to see if they interfere with the fluorescence (see step 8.2). A suggested buffer is sterile tissue culture–grade D-PBS: KCl (200 mg/L), KH_2PO_4 (200 mg/L), NaCl (8,000 mg/L), and Na_2HPO_4 (1150 mg/L).

References

1. Principles and Methods of Toxicology, Third Edition, A.W. Hayes, Ed., Raven Press (1994) pp. 1231–1258; **2.** J Immunol Methods 177, 101 (1994); **3.** J Microbiol Methods 17, 1 (1993); **4.** J Neurosci 15, 5389 (1995); **5.** J Cell Sci 106, 685 (1993); **6.** Pflügers Arch 427, 24 (1994); **7.** Cytometry 20, 181 (1995); **8.** J Biol Chem 270, 23895 (1995); **9.** J Biol Chem 270, 7791 (1995); **10.** J Neurosci 14, 2464 (1994); **11.** J Neurosci 14, 2260 (1994); **12.** J Biol Chem 269, 6803 (1994); **13.** J Cell Biol 133, 1041 (1996); **14.** J Cell Biol 133, 1053 (1996); **15.** J Immunol Methods 177, 101 (1994); **16.** Hum Immunol 37, 264 (1993)

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

Cat #	Product Name	Unit Size
C1430	calcein, AM	1 mg
C3099	calcein, AM *1 mg/mL solution in dry DMSO* *special packaging*	1 mL
C3100	calcein, AM *special packaging*	20 x 50 µg
E1169	ethidium homodimer-1 (EthD-1)	1 mg
L3224	LIVE/DEAD® Viability/Cytotoxicity Kit *for mammalian cells*	1 kit
C36950	CountBright™ absolute counting beads	5 mL

Contact Information

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.

Please visit our website—**probes.invitrogen.com**—for the most up-to-date 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

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
For research use only. Not intended for any animal or human therapeutic or diagnostic use. 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) 335-0504.

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.