BD Pharmingen™ Calcein AM Viability Stain
Live/Dead Cell Discrimination on the BD Accuri™ C6

Features

- Provides high-resolution live/dead cell discrimination using esterase activity
- Expands choice and flexibility in multicolor panel design
- Tested for compatibility with the BD Accuri C6

Table 1. Fluorescence characteristics of BD Pharmingen Calcein AM on the BD Accuri C6.

Characteristic	Calcein AM
Excitation peak	495 nm
Emission peak	515 nm
Laser	488 nm (blue)
Detector	FL1
Equivalent fluorochromes*	FITC, Alexa Fluor® 488

*Do not use these fluorochromes in the same tube with Calcein AM.

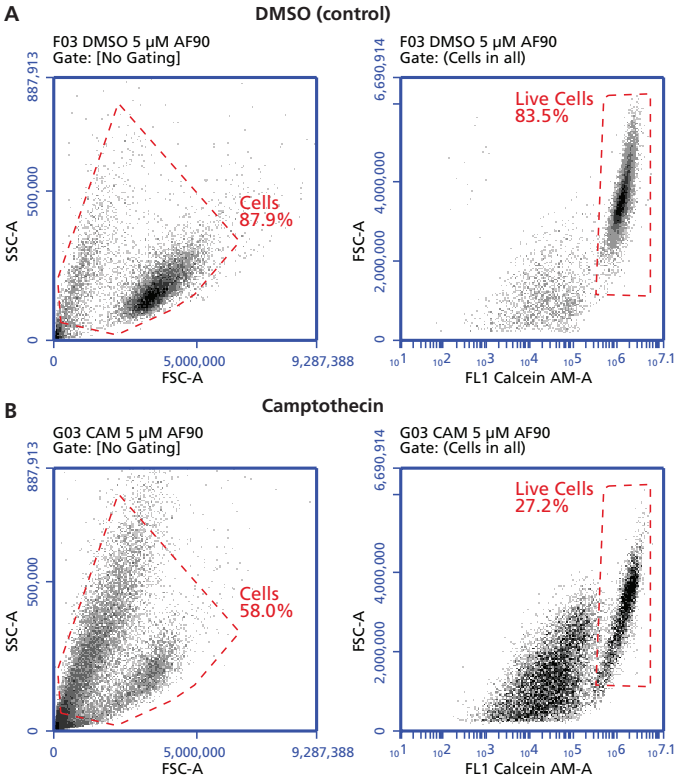

Figure 1. Distinguishing live and dead cells using BD Pharmingen Calcein AM on the BD Accuri C6.

Jurkat cells (Human T-Cell Leukemia; ATCC TIB-152) were treated with 0.025% DMSO (control) or 5 µM of camptothecin for 20 hours to induce apoptosis. The cells were then stained with 5 µM of BD Calcein AM (Cat. No. 564061) in serum-free buffer. Data was acquired on a BD Accuri C6 using an FL1 90% Attenuation Filter (Cat. No. 653173) and analyzed using BD Accuri™ C6 software. **Results:** Cells were initially gated based on light scatter properties (left plots). Live cells were then identified using their Calcein AM fluorescence profiles (right plots). As expected, camptothecin treatment **(B)** dramatically reduced the percentage of live cells compared to controls treated with DMSO **(A)**.

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BD Pharmingen™ Calcein AM can discriminate viable from non-viable cells based on esterase activity. This hydrophobic, non-fluorescent calcein derivative readily diffuses into live cells, where esterases cleave off the acetomethoxy (AM) groups, leaving fluorescent calcein trapped within the cells. Because dead cells lack esterase activity, only viable cells are labeled. Typically, the fluorescence intensity of live cells will be at least ten-fold brighter than that of dead cells. The cells can be further analyzed by flow cytometry or fluorescence imaging.

Table 1 shows the fluorescence characteristics of Calcein AM. Calcein emits bright fluorescence that is detected in the FL1 channel of the BD Accuri™ C6 flow cytometer. This increases flexibility in panel design, since the most common alternatives—the membrane integrity stains propidium iodide (PI) and 7-amino actinomycin D (7-AAD)—are detected in the FL2 and FL3 channels respectively.

Figure 1 shows the use of Calcein AM on the BD Accuri C6 to discriminate live and dead Jurkat cells after treatment with camptothecin vs DMSO controls. For certain cell types, such as the Jurkat cells, titration of Calcein AM (to 5 µM in this case) can reduce the brightness of the signal and aid in visualization on the BD Accuri C6. To further reduce brightness, a BD Accuri™ FL1 90% Attenuation Filter (Cat. No. 653173) was inserted in place of the standard 533/30 FL1 filter.

For other cell types, such as lymphocytes, a higher titer is required to visualize viable cells by calcein fluorescence. In these cases, an attenuation filter must be used. Figure 2 shows peripheral blood mononuclear cells (PBMCs) stained with Calcein AM at three levels of titration. The 10-µM titer achieved the best resolution, but the fluorescence was off scale. Again, the 90% attenuation filter was used to reduce brightness by an order of magnitude, bringing the calcein fluorescence back on scale. The BD Accuri™ FL1 99% Attenuation Filter (Cat. No. 653172) would reduce brightness by an additional order of magnitude.

Easy to use, simple to maintain, and affordable, the BD Accuri C6 personal flow cytometer is equipped with a blue laser, a red laser, two light scatter detectors, and four fluorescence detectors. Compact design, fixed alignment, and pre-optimized detector settings result in a system that is simple to use, and a nonpressurized fluidics system enables kinetic measurements in real time. For walkaway convenience, the optional BD CSampler™ accessory offers automated sampling from 24-tube racks or multiwell plates.

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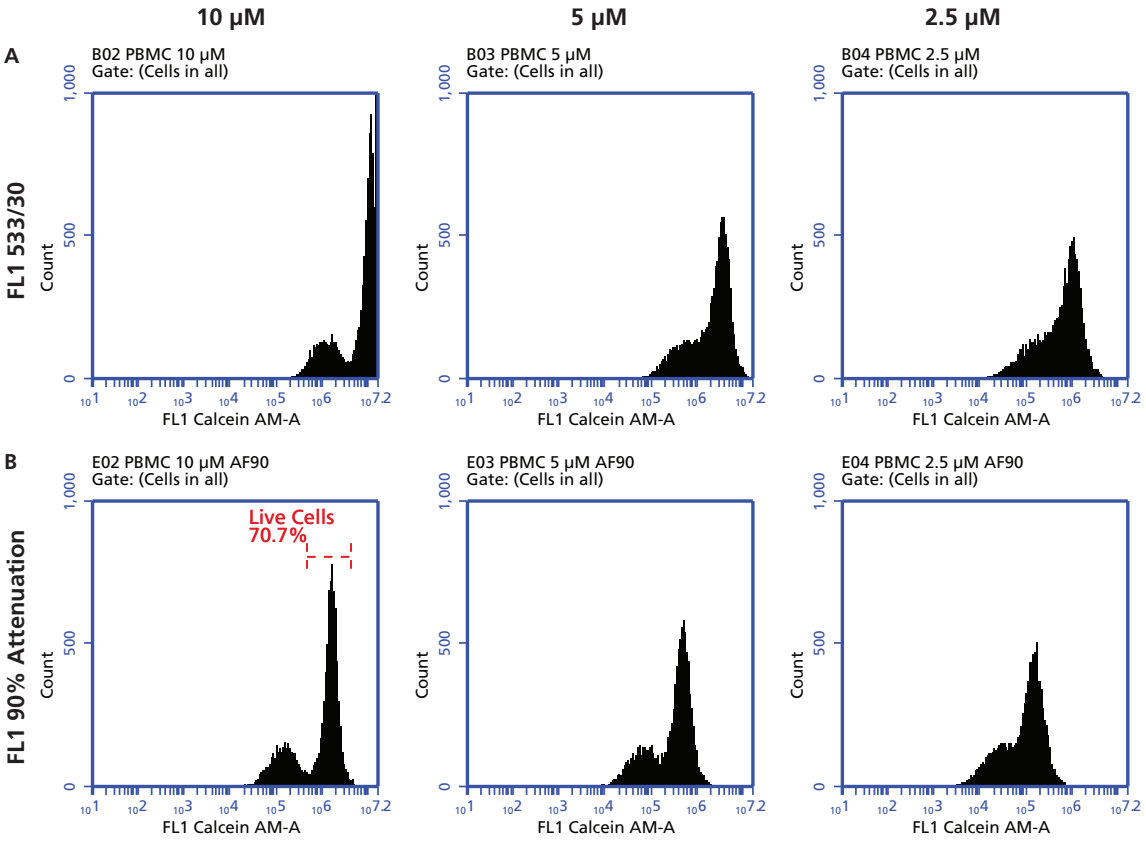

Figure 2. Distinguishing live and dead cells using varying concentrations of BD Pharmingen Calcein AM on the BD Accuri C6.

Human peripheral blood mononuclear cells (PBMCs) were frozen and stored at –80°C for approximately two weeks, resulting in a mixed live/dead population. Cells were thawed and stained with BD Calcein AM (Cat. No. 564061) at concentrations of 10 µM, 5 µM, or 2.5 µM in serum-free buffer. Data was acquired on a BD Accuri C6 flow cytometer with or without the use of an FL1 90% Attenuation Filter (Cat. No. 653173) and analyzed using BD Accuri C6 software. Cells were initially gated based on light scatter properties to identify lymphocytes (not shown). **Results:** Live cells were identified using their Calcein AM fluorescence profiles. The 10-µM titration resulted in the best resolution of live vs dead cells (left plots), but the unattenuated signal **(A)** was off scale. Thus, signal attenuation **(B)** was necessary to visualize calcein fluorescence. Note that plotting Calcein AM signal versus a morphology parameter such as SSC or FSC (not shown) might also help to distinguish live and dead populations, since the morphology of non-viable cells is altered.

Ordering Information

Description	Quantity	Cat.No.
BD Pharmingen™ Calcein AM	1.0 mg	**564061**
BD Accuri™ FL1 90% Attenuation Filter		**653173**
BD Accuri™ FL1 99% Attenuation Filter		**653172**

23-16467-00

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