Technical Data Sheet

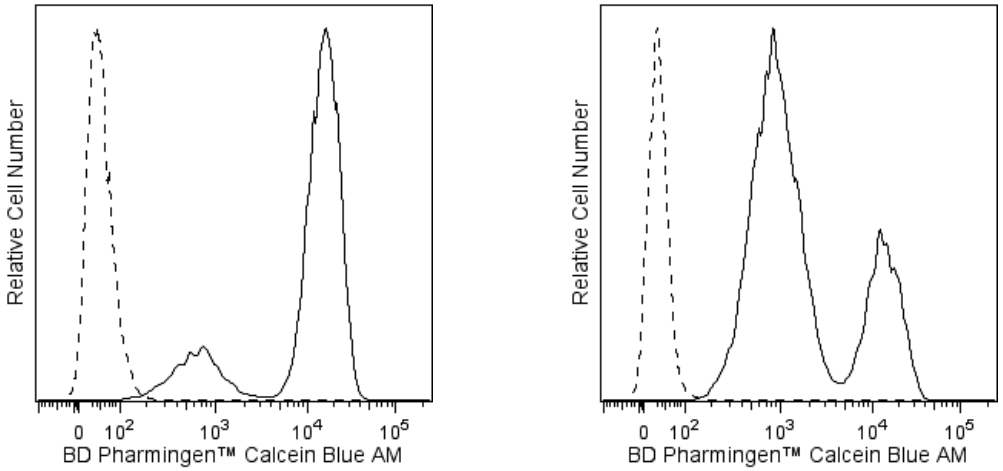
Calcein Blue AM

Product Information

Material Number: **564060**
Size: 1 mg

Description

BD Pharmingen™ Calcein Blue AM is used for labeling live cells that can be detected and further analyzed by flow cytometry. The enhanced hydrophobicity provided by the acetomethoxy (AM) group allows this dye to readily enter viable cells. Once inside, intracellular esterases cleave the AM groups off of the weakly fluorescent Calcein Blue AM, trapping fluorescent Calcein Blue within the cell. Since dead cells lack esterase activity, only viable cells are labeled. Calcein Blue is optimally excited at the 360 nm wavelength of light that can be provided by an ultraviolet laser and emits maximally at 445 nm. This dye can also be excited by the violet laser.



Flow cytometric analysis of Calcein Blue fluorescence in Jurkat cells. *Cells from the human Jurkat (Acute T cell leukemia, ATCC TIB-152) cell line were treated with 0.025% DMSO (Left Panel) or 5 µM camptothecin (Right Panel) for 16 hours and then stained with 10 µM BD Pharmingen™ Calcein Blue AM in serum-free buffer (Cat. No. 564060; solid line histograms). Unstained cells are indicated by the dashed line histograms. Non-viable cells show a decrease in fluorescence intensity. Histograms were derived from gated events with the light scattering characteristics of Jurkat cells. Calcein Blue was excited with a 405 nm wavelength laser light source and detected using a 450/50 nm filter. Flow cytometric analysis was performed using a BD LSRFortessa™ Cell Analyzer System. Calcein Blue AM was also tested on mouse (data not shown).*

Application Notes

Application

Flow cytometry	Tested During Development

Recommended Assay Procedure:

Preparation

Bring BD Pharmingen™ Calcein Blue AM powder and fresh cell culture-grade Dimethyl Sulfoxide (DMSO; e.g., Sigma, Cat. No. D2650) to room temperature. Add 430 µl of DMSO to make a 5 mM stock solution (Molar Mass = 465.41g/mol) and vortex solution well. Inspect the solution and repeat vortex until the stock dye has fully dissolved.

Storage

Store BD Pharmingen™ Calcein Blue AM desiccated at -80°C, protected from light until use. After reconstitution with DMSO, store the solution at -20°C in small aliquots. Avoid freeze-thaw cycles. Please discard the dye aliquots after 6 months post reconstitution with DMSO.

Notes

- When diluted with an aqueous solution, BD Pharmingen™ Calcein Blue AM should be used immediately, as the esterase-cleavable dye may spontaneously hydrolyze with prolonged aqueous exposure.
- Please note that these dyes are esterase cleavable. Serum may have esterase activity, and thus serum-containing buffers may decrease the resolution of viable and non-viable cells if used during staining. Serum-containing buffers may be used for wash steps after staining is complete.
- This dye is not fixable.

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General Procedure

1. Prepare cells for flow cytometric staining using serum-free buffer, (e.g., 1X Dulbecco's Phosphate Buffered Saline).
2. Wash cells one time in serum-free buffer.
3. Resuspend cells at ~1-10 × 10^6 cells/ml in dye diluted in serum-free buffer.
    a. We recommend staining at a 10 µM final concentration for live/dead cell discrimination and multicolor applications as a starting point. Since fluorescence intensity may vary based on cell type and experimental conditions, a titration of the dye is recommended to determine the optimal concentration for each experimental protocol.
4. Incubate the mixture for 30 minutes at room temperature protected from light.
5. Wash cells twice with 2 ml BD Pharmingen™ Stain Buffer (FBS) or the equivalent.
6. Aspirate the supernatant and gently mix to disrupt the cell pellet.
7. Resuspend the cells in Stain Buffer (FBS) or equivalent.
8. Analyze by flow cytometry or stain cells as desired for downstream applications.

Notes on Adherent Cells

Adherent cells may be stained in suspension or *in situ*.

To stain adherent cells in suspension, follow the "General Procedure" as outlined above after cell dissociation into a single cell suspension. Note that for some cells, enzymatic removal from the growth substrate may impact cell membrane integrity, which may affect staining with BD Pharmingen™ Calcein Blue AM. Allowing the cells to recover from the enzymatic dissociation in growth media is recommended. The time of recovery may vary by cell line, so it may be useful to test multiple recovery periods in initial experiments.

To stain *in situ*, we recommend the following protocol:

1. Remove media and wash the cells once with serum-free buffer to remove any remaining media.
2. Add dye diluted in serum-free buffer to the culture.
    a. We recommend staining at a 10 µM final concentration for live/dead cell discrimination and multicolor applications as a starting point. Since brightness may vary based on cell type and experimental conditions, a titration is recommended to determine the optimal concentration for each experimental protocol.
3. Incubate the mixture for 30-60 minutes at 37°C protected from light.
4. Wash cultures twice with BD Pharmingen™ Stain Buffer (FBS) or the equivalent.
5. Remove the cells from the growth substrate. We recommend using BD™ Accutase™ Cell Detachment Solution.
    a. Wash cells twice with BD Pharmingen™ Stain Buffer (FBS) or the equivalent.
    b. Resuspend the cells in Stain Buffer (FBS) or equivalent.
    c. Analyze by flow cytometry or stain cells as desired for downstream applications.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
561527	Accutase™ Cell Detachment Solution	100 mL	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
3. Accutase is a registered trademark of Innovative Cell Technologies, Inc.
4. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
5. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Coder DM. Assessment of cell viability. *Curr Protoc Cytom.* 2013; Ch. 9(Unit 9.2):1-26. (Methodology)

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Giordano G, Hong S, Faustman EM, Costa LG. Measurements of cell death in neuronal and glial cells. *Methods Mol Biol.* 2011; 758:171-178. (Biology)

Huitink GM, Poe DP, Diehl H. On the properties of Calcein Blue. *Talanta.* 1974; 21(12):1221-1229. (Biology)