Technical Data Sheet

Fluo-4 AM

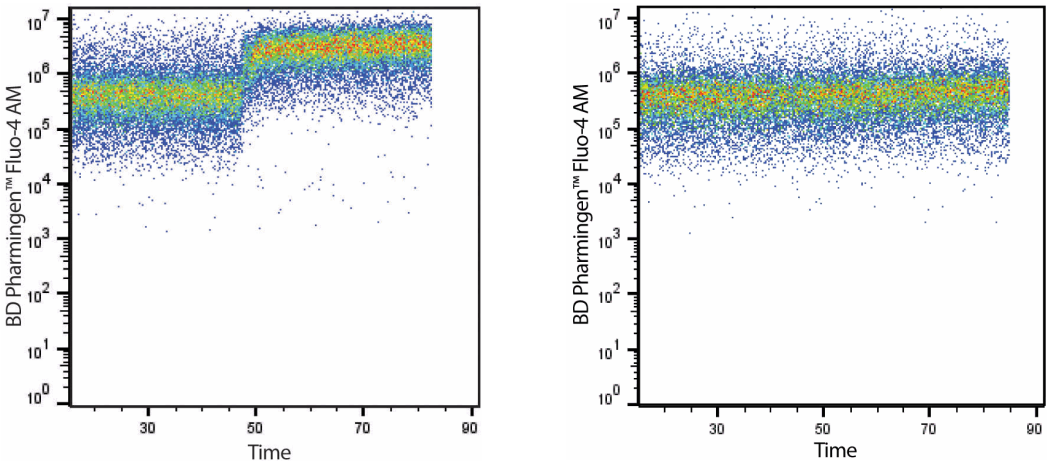
Product Information

Material Number:	**565878**
Size:	0.5 mg
Component:	**51-9011906**
Description:	Fluo-4 AM
Size:	50 ug (10 ea)

Description

BD Pharmingen™ Fluo-4 AM is a cell-permeant dye that can be used to measure the intracellular ionized calcium concentration, [Ca2+]i. The hydrophobic acetomethoxy (AM) moiety allows passage across the cell membrane into viable cells. Once inside, intracellular esterases cleave the AM groups, leaving Fluo-4 trapped with the cell and free to bind intracellular calcium. The Kd for Ca2+ is approximately 335 nM in physiologic buffers.

Calcium-bound BD Pharmingen™ Fluo-4 AM has an excitation maximum of 494 nm and an emission maximum of 506 nm.



Intracellular calcium response of Jurkat cells to stimulation with A23187. *Jurkat cells were stained with 5 µM BD Pharmingen™ Fluo-4 AM (Cat. No. 565878) in DPBS with calcium and magnesium for 30 minutes at 37°C. Cells were pelleted once and further incubated in complete medium for 30 minutes to allow complete hydrolysis of AM moieties. Cells were then pelleted and resuspended in DPBS with calcium and magnesium and analyzed on an BD Accuri™ C6 in the FL-1 channel. After 30 seconds of acquisition, 1 µM calcium ionophore A23187 (left, Sigma Aldrich Cat. No. C7522) or DMSO vehicle (right) was added to the tube, followed by mixing with the pipette. Jurkat cells show an increase in intracellular calcium concentration with addition of calcium ionophore A23187, but not with addition of DMSO vehicle.*

Application Notes

Application

Flow cytometry	Tested During Development

Recommended Assay Procedure:

Preparation

Bring Fluo-4 AM dye powder and anhydrous Dimethyl Sulfoxide to room temperature. Add 9 µl of DMSO to dye powder and vortex solution well. Inspect the solution and repeat vortex until the stock dye has fully dissolved. This yields a 5 mM stock solution.

Storage

Upon arrival, store the dry dye desiccated and protected from light at -20°C until use. We recommend a fresh vial of dye be used for each experiment and that reconstituted dye be discarded after use. However, if stock solutions are to be kept for use, they should be stored desiccated and protected from light at -20°C and used within one week of reconstitution.

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Cytometry Requirements

Blue (eg, 488 nm) laser-equipped flow cytometers (eg, BD Accuri™ C6, BD LSRFortessa™, BD LSRFortessa™ X-20, or BD™ LSR II) can be used. This dye can be read out of filters commonly used for FITC or Alexa Fluor® 488 (eg, 530/30).

Fluorescence compensation is best achieved using a sample of the cells of interest stained with the dye. When designing multicolor panels, please be aware of spillover into the PE and BD Horizon™ PE-CF594 channels on the blue laser. If available, collecting these fluorochromes using the yellow-green (eg, 561 nm) laser may be advantageous to avoid spillover from Fluo-4 AM. Panels should be optimized to take this spillover into account.

Procedure

BD Pharmingen™ Fluo-4 AM Labeling of Cells

1. Prepare a single cell suspension at 1×10e6 cells/mL in physiologic loading buffer of choice.
    - If serum is used in the loading buffer, it should be heat inactivated in order to prevent residual serum esterase activity from cleaving AM moieties on the dye prior to entry into cells.
2. Add dye stock solution for a final staining concentration of 1 - 5 µM and vortex immediately.
    - We recommend using the lowest dye concentration that still yields sufficient signal in order to avoid dye toxicity, compartmentalization, and calcium buffering.
3. Incubate 15 - 60 minutes at 37°C.
    - It is reported that dye compartmentalization is less significant at lower loading temperatures. In this case, it may be advantageous to load cells at room temperature if dye compartmentalization is significant for the cell type of interest.
4. Wash once and resuspend in analysis buffer of choice.
    - For some cell types, it may be advantageous to incubate cells for another 30 minutes to allow complete de-esterification of AM moieties. In this case, cells should be incubated in physiologic buffer of choice or complete medium, washed once more, and then resuspended in analysis buffer of choice.
5. Proceed to flow cytometry.

Product Notices

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

References

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