VFSE™ 500

Ordering Information	*Storage Conditions*
Product Number: 22024	Keep at -20 °C and desiccated Expiration date is 12 months from the date of receipt

Introduction

It is widely recognized that fluorescent labeling of cells is an effective method for detecting the presence of viable cells in a sample. Flow cytometry combined with fluorescent staining is a powerful tool to analyze heterogeneous cell populations. Among all the existing fluorescent dyes, fluorescein diacetate (FDA) and its derivatives (such as CFSE) are non-fluorescent molecules that diffuse into cells and are hydrolyzed by intracellular non-specific esterases to give fluorescent products. The fluorescent products are generated and accumulated only in the cells that have intact cell membranes and active esterase activities while dead cells are not stained. The precise kinetics of membrane transport and intracellular hydrolysis of FDA and its analogs (such as CFSE) are related to cellular functions. However, it is impossible to use CFSE and its fluorescein analogs for GFP-transfected cells or for the applications where a FITC-labeled antibody is used since CFSE and its fluorescein analogs have the excitation and emission spectra almost identical to GFP or FITC. VFSE™ dyes are functionally similar to CFSE since they contain both an esterase-cleavable and an amine-reactive SE group. VFSE™ dyes can be used for the multicolor applications where either GFP or FITC-labeled antibody is used since VFSE™ dyes have either excitation or emission spectra distinct from CFSE and its fluorescein analogs.

Chemical and Physical Properties

Molecular Weight: 531.96
Solvent: Dimethylsulfoxide (DMSO)
Spectral Properties: Ex/Em = 410/500 nm

Assay Protocol with VFSE™ 500

Brief Summary

Prepare cells with test compounds → Add VFSE™ 500 working solution (1 to 25 µM) → Incubate at room temperature or 37 °C for 5 to 15 min → Remove the VFSE™ 500 working solution → Read fluorescence intensity at Ex/Em = 405/500 nm

Note: Following is our recommended protocol for live cells. This protocol only provides a guideline, and should be modified according to your specific needs.

1. Prepare VFSE™ 500 working solution:

1.1 Prepare a 2 to 10 mM stock solution of VFSE™ 500 in high-quality, anhydrous DMSO. The stock solution should be used promptly; any remaining solution should be aliquoted and refrozen at ≤ -20 °C.
Note: Avoid repeated freeze-thaw cycles, and protect from light.

1.2 Prepare a 1 to 25 µM working solution of 1X VFSE™ 500 right before use by diluting VFSE™ 500 DMSO stock solution (from Step 1.1) in Hanks and 20 mM Hepes buffer (HHBS) or buffer of your choice, pH 7 with 0.02% Pluronic® F-127. Mix them well by votexing.

2. Run VFSE™ 500 assay with a fluorescence microscope or a flow cytometer:

2.1 Treat cells with test compounds for a desired period of time.
2.2 Centrifuge the cells to get 1-5 × 10^5 cells per tube.
2.3 Resuspend cells in 500 µL of VFSE™ 500 working solution (from Step 1.2).

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2.4 Incubate at room temperature or 37 °C for 5 to 10 min, protected from light.
2.5 Remove the VFSE™ 500 working solution from the cells, wash the cells with HHBS or buffer of your choice. Resuspend cells in 500 µL of prewarmed HHBS or medium to get 1-5 × 10^5 cells per tube.
2.6 Monitor the fluorescence change at Ex/Em = 405/500 nm with a fluorescence microscope or a flow cytometer.

3. Run VFSE™ 500 assay with a fluorescence plate reader or a fluorescence microscope:

3.1 Treat cells with test compounds for a desired period of time.
3.2 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of VFSE™ 500 working solution (from Step 1.2) into the cell plate.
3.3 Incubate the VFSE™ 500 loading plate in a 37 °C and 5% CO_2 incubator for 15-60 min.
Note: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.
3.4 Remove the VFSE™ 500 working solution from the plate, wash the cells with HHBS or buffer of your choice. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of HHBS back to the cell plate.
3.5 Monitor the fluorescence change at Ex/Em = 405/500 nm with a fluorescence microplate reader or a fluorescence microscope.

Disclaimer: *This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact our technical service representative for more information.*

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