

VFSE™ 550

Ordering Information

Product Number: 22027

Storage Conditions

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 monitoring 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: 451.86
Solvent: Dimethylsulfoxide (DMSO)
Spectral Properties: Ex/Em = 398/546 nm

Assay Protocol with VFSE™ 550

Brief Summary

Prepare cells with test compounds → Add 1 to 25 µM VFSE™ 550 working solution → Incubate at room temperature or 37 °C for 5 to 15 min → Remove the VFSE™ 550 working solution → Read fluorescence intensity at Ex/Em = 405/550 nm

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

1. Prepare VFSE™ 550 working solution:

- 1.1 Prepare a 2 to 10 mM stock solution of VFSE™ 550 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™ 550 right before use by diluting VFSE™ 550 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 vortexing.

2. Run VFSE™ 550 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 $1-5 \times 10^5$ cells per tube.
- 2.3 Resuspend cells in 500 μ L of VFSE™ 550 working solution (from Step 1.2).
- 2.4 Incubate at room temperature or 37 °C for 5 to 10 min, protected from light.
- 2.5 Remove the VFSE™ 550 working solution from the cells, wash the cells with HHBS or buffer of your choice. Resuspend cells in 500 μ L of prewarmed HHBS or medium to get $1-5 \times 10^5$ cells per tube.
- 2.6 Monitor the fluorescence change at Ex/Em = 405/550 nm with a fluorescence microscope or a flow cytometer.

3. Run VFSE™ 550 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 μ L/well (96-well plate) or 25 μ L/well (384-well plate) of VFSE™ 550 working solution (from Step 1.2) into the cell plate.
- 3.3 Incubate the VFSE™ 550 loading plate in a 37 °C, 5% CO₂ 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™ 550 working solution from the plate, wash the cells with HHBS or buffer of your choice. Add 100 μ L/well (96-well plate) or 25 μ L/well (384-well plate) of HHBS back to the cell plate.
- 3.5 Monitor the fluorescence change at Ex/Em = 405/550 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.