Product Information Sheet

Fluorescein di-O-acetate (FDA) (product #0060)

Structure:

\[
\begin{array}{c}
\text{O} \\
\text{CH}_3\text{C-O} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{O} \\
\text{C-C} \\
\end{array}
\]

MW: 416

Storage: freeze, dessicate

Soluble: DMSO, Acetone

Abs: < 300

Em: none

Note: converted to fluorescein upon acetate hydrolysis (Abs: 490 nm, E: 90 K, Em: 514 nm)

Application Information

NOTE: The following information is given as possible methodology for use of FDA in cell viability assays. The user may determine their own best conditions for use dependent on the specific conditions present in their experiment.

I. OVERVIEW

Viable cells, both mammalian and, in many cases, bacterial (1), have the capability to incorporate the nonpolar, nonfluorescent compound fluorescein di-O-acetate (FDA) and rapidly hydrolyze it using acetyl esterase activity to fluorescein, a polar, fluorescent compound which is retained within the cell. Nonviable cells no longer have esterase activity and will not be fluorescently stained. Furthermore, nonviable cells are susceptible to DNA staining with compounds such as ethidium bromide or propidium iodide, and can therefore be easily counterstained to differentiate them from viable cells in a fluorometric assay.
On the basis of these principals, FDA can be easily used in a double staining procedure in combination with propidium idodide to determine cell viability in cell suspension. After staining, cell viability can be assayed using slide preparations or FACS analysis (2, 3). Viable cells fluoresce bright green, while nonviable cells are bright red.

II. MATERIALS AND METHODS

A.) Stock Solutions. A stock solution of 5mg/ml FDA can be prepared using acetone or DMSO. This solution should be stored at -20°C. Stock solutions of propidium iodide (PI) may be prepared in phosphate buffered saline (PBS).

B.) Staining. Staining of mammalian cells in suspension (2, 3). Cells should be suspended in PBS or HBSS and kept on ice. Staining is performed with final concentrations of FDA from .5 - 10 µg/ml, and PI from 3 - 50 µg/ml. A ratio of 1x10^6 cells per 1 µg FDA and .3 µg PI works well. After incubation at room temperature for up to 30 min, cells should be placed on ice until analysis. Cells may then be analyzed using FACS or slide preparations.

Staining of bacterial cells (1): Staining is most efficient when stock solution is diluted 1:800 in nutrient broth preconditioned by overnight growth of the test bacteria, but fresh nutrient broth or PBS may also be used. Bacterial suspensions should be diluted with PBS to 10^7 organisms per ml. Bacteria may be stained by applying one ml of solution to .45 µm filter (25mm) and vacuum filtering to remove solution, then adding 1ml of FDA solution and incubating 5 - 10 minutes at room temperature.

III. REFERENCES

