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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

CytoTell™ Multi-Color Cell Proliferation Assay Panel

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, CFSE is frequently used. The non-fluorescent CFSE molecule diffuses into cells and is hydrolyzed by intracellular non-specific esterases to give the highly fluorescent fluorescein product. The fluorescent product is 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 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. Our CytoTell™ dyes are functionally similar to CFSE and can be used for the multicolor applications where either GFP or FITC-labeled antibody is used since the CytoTell™ dyes have either excitation or emission spectra distinct from CFSE and its fluorescein analogs. Moreover, our CytoTell™ dyes not only eliminates the dye efflux drawback associated with CFSE, but also is compatible with cell culture medium in the staining cells prior to imaging or flow cytometric analysis.

Spectral Properties of CytoTell™ Cell Proliferation Dyes

AAT Bioquest offers CytoTell™ dyes that enable the multicolor labeling and functional analysis of live cells in combination with CFSE. They are optimized for the excitation wavelengths of a variety of flow cytometers, providing additional colors for flow cytometric analysis of live cells.

Table 1. Fluorescence spectra properties and suggested excitation laser for flow cytometry analysis.

Product Number	Indicator	Size	Form	Ex/Em (nm)	Excitation Source
22240	CytoTell™ UltraGreen	500 tests	Powder (1 vial)	492/519	488 nm (Blue Laser)
22241	CytoTell™ UltraGreen	1000 tests	Powder (2 vials)	492/519	488 nm (Blue Laser)
22251	CytoTell™ Blue	500 tests	Powder (1 vial)	403/454	405 nm (Violet Laser)
22252	CytoTell™ Blue	1000 tests	Powder (2 vials)	403/454	405 nm (Violet Laser)
22253	CytoTell™ Green	500 tests	Powder (1 vial)	511/525	488 nm (Blue Laser)
22254	CytoTell™ Green	1000 tests	Powder (2 vials)	511/525	488 nm (Blue Laser)
22255	CytoTell™ Red 650	500 tests	Powder (1 vial)	628/643	633 nm (Red Laser)
22256	CytoTell™ Red 650	1000 tests	Powder (2 vial)	628/643	633 nm (Red Laser)
22257	CytoTell™ Orange	500 tests	Powder (1 vial)	542 /556	488 nm (Blue Laser) 531 nm (Green Laser)
22258	CytoTell™ Orange	1000 tests	Powder (2 vials)	542 /556	488 nm (Blue Laser) 531 nm (Green Laser)
22261	CytoTell™ Red 590	500 tests	Powder (1 vial)	560 /574	488 nm (Blue Laser) 531 nm (Green Laser)
22262	CytoTell™ Red 590	1000 tests	Powder (2 vials)	560 /574	488 nm (Blue Laser) 531 nm (Green Laser)

Storage and Handling Conditions

The CytoTell™ dyes are lyophilized powders. They should be stable for at least 6 months if store at -20 °C, protecting from light, and avoiding freeze/thaw cycles.

Assay Protocol

Brief Summary

Prepare cells with test compounds → Add 1X dye working solution → Incubate dyes with cells at RT or 37 °C for 10 to 30 min → Remove the dye working solution → Analyze with a flow cytometer

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

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

1. Prepare 500 X DMSO stock solution

Add 500 µL DMSO into the dye powder vial, mix it well by vortexing to have a 500X DMSO stock solution
Note: The stock solution should be used promptly; any remaining solution should be aliquoted and frozen at ≤ -20 °C. Avoid repeated freeze-thaw cycles, and protect from light.

2. Prepare 1X dye working solution

Prepare a 1X dye working solution by diluting the 500X DMSO stock solution at 1 to 500 in Hanks and 20 mM Hepes buffer (HHBS) or the buffer of your choice, pH 7 (such as 1 µL of 500X DMSO stock solution to 500 µL buffer) right before use. Mix them well by vortexing.
Note: The final concentration of the dye working solution should be empirically determined for different cell types and/or experimental conditions. It is recommended to test at the concentrations that are at least over a t en fold range. Such as CytoTell™ Red might use much less amount in some cell types than the recommend concentrations.

3. Analyze cells with a flow cytometer or a fluorescence microscope:

3.1 Treat cells with test compounds for a desired period of time.

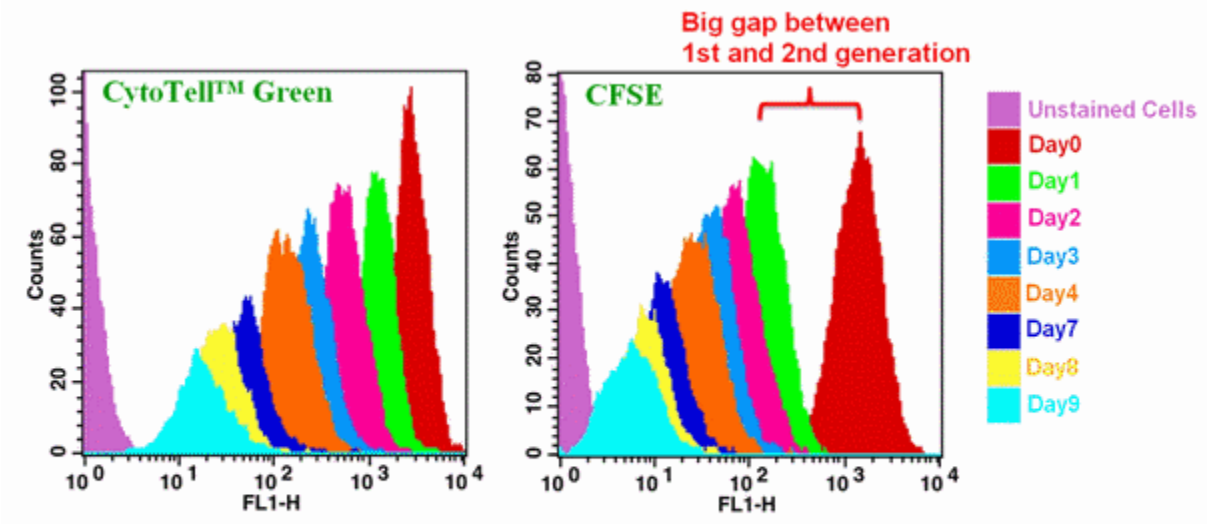
3.2 Centrifuge the cells to get 1-5 × 10^5 cells per tube.

3.3 Resuspend cells in 500 µL of the dye working solution (from Step 2).
Optional: One can add the 500X DMSO stock solution into the cells directly without medium removing (such as, add 1 µL500X DMSO stock solution into 500 µL cells)

3.4 Incubate cells with a dye solution at room temperature or 37 °C for 10 to 30 min, protected from light.

3.5 Remove the dye working solution from the cells, wash the cells with HHBS or buffer of your choice. Resuspend cells in 500 µL of pre-warmed HHBS or medium to get 1-5 × 10^5 cells per tube.

3.6 Monitor the fluorescence change at respected Ex/Em (see Table 1) with a flow cytometer or a fluorescence microscope.



Cell tracking assay with CytoTell™ Green and CFSE. Jurkat cells (~2×10⁶ cells/mL) were stained with CytoTell ™Green or CFSE (0.5µM) on Day0. The cells were passed serially at 1:1 ratio for 9 days. Fluorescence intensity was measured with FACS Calibur flow cytometer in FL1 channel on the day after passage. Successive generations were represented by different colors.

Disclaimer: *These products are for research use only and are not intended for therapeutic or diagnostic applications. Please contact our technical service representative for more information.*