CFSE Cell Division Assay Kit

Item No. 10009853

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. Once opened, store individual components as stated below.

Item Number	Item	100 Tests Quantity/ Size	Storage
600121	CFSE Stock Solution	3 vials/100 µl	-20°C
10009322	Cell-Based Assay Buffer Tablet	4 tablets	Room temperature

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Adjustable pipettes and a repeat pipettor
2. 6-, 12-, or 24-well plates for culturing cells
3. Freshly isolated human peripheral blood mononuclear cells (PBMC), or cells of your choice
4. A flow cytometer equipped with a 488 nm excitation laser
5. Reagents, such as Ficoll-Paque Plus (Amersham Biosciences, Item No. 17-1440-02), used to isolate lymphocytes from peripheral blood cells
6. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

Cell proliferation is controlled by growth factors that bind to receptors on the cell surface, which in turn connect to signaling molecules. These molecules activate transcription factors which bind to DNA to turn on or off the production of proteins which result in cell division. Dysfunction of any step in this regulatory cascade causes abnormal cell proliferation, which underlies many human pathological conditions.[1] The mechanisms underlying alterations in cell cycle progression are crucial to understanding many human diseases, most notably cancer.

Carboxyfluorescein diacetate, succinimidyl ester (CFDA-SE) is a novel cell-tracing fluorescent dye used to examine the proliferative activity of cells by the labeling of a parent generation and the inheritance of the label by daughter generations. CFDA-SE diffuses into cells, where the acetate groups on the molecule are cleaved to yield a highly fluorescent derivative (CFSE) that is retained in the cell and can be detected by flow cytometry. Cell division results in sequential halving of fluorescence, and up to eight divisions can be monitored before the fluorescence is decreased to the background fluorescence of unstained cells.[2] In addition, the stability of the CFSE labeling allows monitoring of cells over a period of a couple of weeks *in vitro*. Using this method, cells may be traced through successive generations without the use of radioactivity and complicated protocols. Since its first introduction in 1994, the flow cytometry analysis of CFSE-labeled cells has become the most informative experimental techniques for studying lymphocyte proliferation.[3]

About This Assay

Cayman's CFSE Cell Division Assay Kit provides an easy to use format for labeling and tracing cells through successive cell divisions which can be used to study the induction and inhibition of cell division in any *in vitro* model. The kit contains sufficient reagents for labeling and analyzing 100 cell samples by flow cytometry. CFSE can also be combined with any fluorochrome compatible with fluorescein for use in flow cytometry.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

1. Assay Buffer Preparation

Dissolve each Cell-Based Assay Buffer Tablet (Item No. 10009322) with 100 ml of distilled water. Mix well to ensure that the tablet dissolves completely. Filter the buffer through a 0.2 µm filter. The buffer is stable for one year at room temperature.

2. CFSE Staining Solution Preparation (2X)

Prepare a CFSE Staining Solution by diluting the CFSE Stock Solution (Item No. 600121) 1:200 with the Assay Buffer prepared above. The 2X CFSE Staining Solution is stable for one hour at room temperature. *Protect from light!*

Performing the Assay

General Information

- To use freshly isolated blood monocytes, collect blood into heparinized tubes. Enrich lymphocytes using a Ficoll-Paque Plus gradient, as described in the manufacturer's protocol (Amersham Biosciences Item No. 17-1440-02). Alternatively, isolate lymphocytes according to your typical experimental protocol. Label cells with CFSE by following the CFSE Labeling of Cells procedure.
- To use cells other than freshly isolated blood monocytes, prepare cells according to your typical experimental protocol. Label the cells with CFSE by following the CFSE Labeling of Cells procedure.
- If you are using cell lines, you are more likely to see less than 3 peaks since the broad level of CFSE staining on the cell lines makes resolution of divisions difficult.
- It is critical to include a control (cells treated with vehicles) for determining an undivided peak. The control must be incubated for the same duration as your labeled cells. It is also important to include non-CFSE-labeled cells for determining the autofluorescence level of divided cells.

CFSE Labeling of Cells

1. Centrifuge the cells at 1,250 rpm for five minutes at room temperature to pellet the cells.
2. Wash cells with pre-warmed sterile PBS and centrifuge as above.
3. Resuspend cell pellet in pre-warmed PBS at a concentration of 5×10^6-1×10^7 cells/ml.
4. Add an equal volume of 2X CFSE Staining Solution prepared above to the cell mixture from step 3. The final dilution of CFSE is 1:400.
5. Incubate the cells at 37°C, 5% CO_2 for 30 minutes. Invert tubes occasionally to make sure there is even labeling of the cells with CFSE.
6. Centrifuge the cells at 1,250 rpm for five minutes at room temperature to pellet the cells.
7. Aspirate the supernatant and resuspend the cells in culture medium containing 10% FBS.
8. Incubate the cells at 37°C, 5% CO_2, for 30 minutes. Invert tubes occasionally to make sure the cells are well suspended.
9. Pipette gently to uniformly suspend cells. Perform a cell count using a hemocytometer.
10. Centrifuge the cells at 1,250 rpm for five minutes at room temperature to pellet the cells. At this point, stain cells for other cell surface markers such as CD3 if appropriate. Otherwise go directly to the next step.
11. Determine CFSE staining (and percentage of other cell surface marker staining if applicable) by flow cytometry.
12. Adjust cell number/ml to the concentration required for your assay. The cells are now ready for use in your experiment

Assay Procedure

1. Plate the CFSE-labeled cells prepared from above in a 6-, 12-, 24-, or 96-well plate at a density of 1 x 10^6-5 x 10^6 cells/well.
2. Add experimental compounds or vehicle to each well to a final concentration according to your experimental protocol. If you are testing the stimulatory or inhibitory effect of certain cell types on the CFSE-labeled lymphocytes, add the cells to each well containing CFSE-labeled cells to a ratio appropriate for your experimental protocol.
3. Culture the cells for four to seven days in 37°C, 5% CO_2 incubator, or for a period of time according to your typical experimental protocol. Take samples at time intervals for analysis using a flow cytometer with excitation at 488 nm and an emission filter used for fluorescein, usually the FL1 channel of a cytometer.
4. If the cells are to be cultured for more than four days, replace the culture medium with fresh medium as needed.
5. At the end of the experiment, collect cells into tubes and centrifuge at 1,250 rpm at room temperature for five minutes.
6. Wash the cells one time with PBS.
7. Resuspend the cells in PBS. Mix well to ensure mono-dispersion of the cells.
8. Analyze the cells using a flow cytometer with excitation at 488 nm and an emission filter used for fluorescein, usually the FL1 channel of a cytometer.

ANALYSIS

Performance Characteristics

An example of typical data obtained using flow cytometry is shown in the figure below. Your results will vary based on the cell type and experimental protocol used.

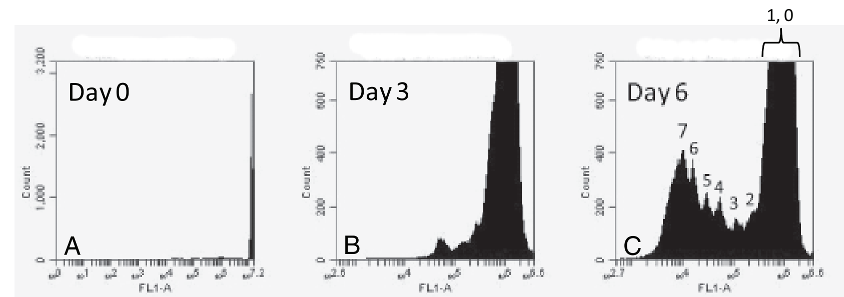

Figure 1: BDCM (a human DC-like cell line which can be obtained from ATCC) stimulates T-cell proliferation when the cells are co-cultured together for six days. Human peripheral blood lymphocytes isolated from freshly collected blood were labeled with CFSE on Day 0. CFSE-labeled lymphocytes were then co-cultured with BDCM cells at a ratio of 25:1 in 2 ml of RPMI culture medium in a 6-well plate for three or six days. *Panel A:* CFSE fluorescence intensity is strong at the time of staining (Day 0). *Panel B and C:* CFSE staining intensity drops rapidly in the first couple of days due to catabolism. As cell division occurs, the staining intensity stabilized (Day 3 and Day 6). *Panel C:* Eight peaks representing successive cell cycles of lymphocytes were detected after six days of BDCM stimulation (the first peak shown here actually contains two peaks representing undivided cells, peak 0, and first division cells, peak 1).

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Low signal of CFSE	A. Cells not healthy B. Cells not well labeled by CFSE	A. Use only healthy cells B. Perform a titration of CFSE to get an optimal concentration of CFSE staining
Cell death during process	A. CFSE concentration is too high, resulting in cytotoxicity B. The concentration of the experimental compound is too high, resulting in cytotoxicity	A. Perform a titration of CFSE to get an optimal concentration of CFSE B. Lower concentration of the experimental compound

References

1. Šulic, S., Panic, L., Đikic, I., *et al.* Deregulation of cell growth and malignant transformation. *Croat. Med. J.* **46(4)**, 622-638 (2005).
2. Parish, C.R., Glidden, M.H., Quah, B.J.C., et al. Use of the intracellular fluorescent dye CFSE to monitor lymphocyte migration and proliferation. Current Protocols in Immunology 4.9.1-4.9.13 (2009).
3. Lyons, A.B. Analysing cell division *in vivo* and *in vitro* using flow cytometric measurement of CFSE dye dilution. *J. Immunol. Methods* **243**, 147-154 (2000).

Related Products

7-AAD Cell Viability Assay Kit - Item No. 10009856
7-AAD/CFSE Cell-Mediated Cytotoxicity Assay Kit - Item No. 600120
Apoptotic Blebs Assay Kit - Item No. 10010750
Caspase-3 Fluorescence Assay Kit - Item No. 10009135
Cell Cycle Phase Determination Kit - Item No. 10009349
JC-1 Mitochondrial Membrane Potential Assay Kit - Item No. 10009172
LDH Cytotoxicity Assay Kit - Item No. 10008882
Lysosome/Cytotoxicity Dual Staining Kit - Item No. 600310
MTT Cell Proliferation Kit - Item No. 10009365
20S Proteasome Assay Kit - Item No. 10008041
WST-1 Cell Proliferation Assay Kit - Cat No. 10008883
WST-8 Cell Proliferation Assay Kit - Cat No. 10010199
XTT Cell Proliferation Assay Kit - Cat No. 10010200

Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.

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NOTES

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