CFSE Cell Division Assay Kit

Item No. 10009853

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION
- 3 Materials Supplied
- 3 Safety Data
- 4 Precautions
- 4 If You Have Problems
- 4 Storage and Stability
- 4 Materials Needed but Not Supplied

INTRODUCTION
- 5 About This Assay

ASSAY PROTOCOL
- 6 Preparation of Labeling Reagents
- 7 Performing the Assay

ANALYSIS
- 9 Performance Characteristics

RESOURCES
- 10 Troubleshooting
- 10 References
- 11 Notes
- 11 Warranty and Limitation of Remedy

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	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

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. Cells to be CFSE-labeled, such as freshly isolated human peripheral blood mononuclear cells (PBMC) or murine splenocytes.
2. Medium for culturing cells and proliferative stimuli.
3. A flow cytometer equipped with a 488 nm excitation laser.
4. A source of pure water; glass distilled water or HPLC-grade water is acceptable.

INTRODUCTION

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. Carboxyfluorescein diacetate, succinimidyl ester (CFDA-SE) diffuses into cells, where the acetate groups 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.[1,2] The ease of use and lack of cytotoxicity allows monitoring of cellular division over weeks either *in vitro* or *in vivo*. The kit contains sufficient reagents for labeling and analyzing up to 7.5×10^8 cells by flow cytometry. CFSE-labeled cells can be further stained with any fluorochrome compatible with fluorescein for use in flow cytometry.

ASSAY PROTOCOL

Preparation of Labeling 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. **2X CFSE Staining Solution Preparation**

   Prepare a 2X CFSE Staining Solution by diluting the CFSE Stock Solution (Item No. 600121) 1:200 with Assay Buffer. You will need approximately 500 µl per 1-2 x 10^7 cells. This 2X CFSE Staining Solution is stable for one hour at room temperature. *Protect from light! If multiple uses of the CFSE stock solution are anticipated, make smaller aliquots and store at -20°C to minimize freeze-thaw cycles.*

Performing the Assay

Cells for Labeling

- To use freshly isolated blood leukocytes, 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). To use fresh murine splenocytes, dissociate a spleen through 70 µm mesh into a single cell suspension and lyse red blood cells.
- Most cell lines are not likely to yield multiple peaks without treatments that impact cell division, as they are more clonal in nature and tend to divide at the same rate.

Important Controls

- It is critical to include a control for determining an undivided peak. The control must be as labeled with the cells expected to undergo division, as every labeling reaction will be slightly different. It is also helpful to include unlabeled cells for determining the autofluorescence level of divided cells and to include in compensation controls.

CFSE Labeling of Cells

1. Centrifuge the cells at 300 x g for five minutes at room temperature and aspirate the supernatant.
2. Wash cells with pre-warmed sterile PBS and centrifuge as above.
3. Resuspend cell pellet in pre-warmed PBS at a concentration of 1-2 x 10^7 cells/ml.
4. Add an equal volume of 2X CFSE Staining Solution prepared above to the cell suspension, mixing rapidly and thoroughly. The final dilution of CFSE is 1:400.
5. Incubate the cells in a 37°C water bath for 15 minutes. Invert tubes occasionally to make sure there is even labeling of the cells with CFSE.
6. Add at least an equal volume of culture media containing 10% FBS and centrifuge the cells at 300 x g for five minutes at room temperature.
7. Aspirate the supernatant and wash the cells two more times in 10-20 ml of culture medium. After the third centrifugation, resuspend cells to the concentration required for your assay. *NOTE: CFSE labeling can be assessed by flow cytometry at this stage, however readings within 24 hours of labeling should only be used to determine whether or not labeling was successful.*
8. Stimulate cells as your experiment requires, for between 2 and 7 days. Experimental conditions are highly dependent on cells and stimuli used, and so need to be determined separately for every experimental setup.
9. To assay the dilution of CFSE, harvest cells into FACS tubes or wells of a v-bottom plate, wash and read on a flow cytometer with excitation at 488 nm and emission at 525 nm. Additional surface and intracellular staining may be performed without loss of signal, though appropriate single-stained controls must be included to perform compensation.

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.

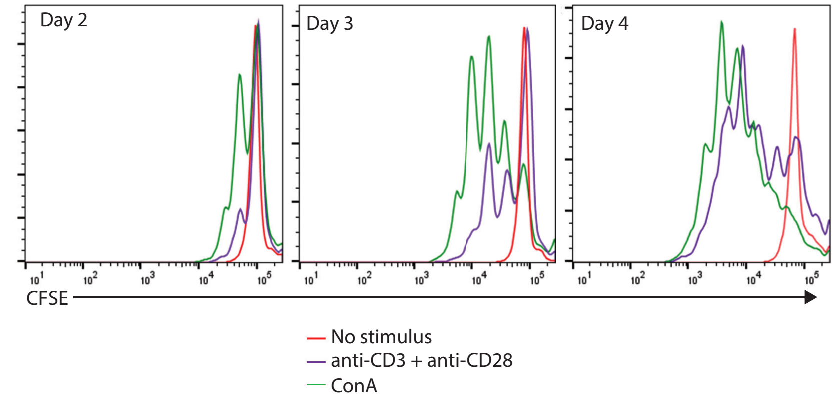

Figure 1: CD4+ T cells proliferate in response to T cell receptor antibodies and mitogenic stimuli. Murine splenocytes were labeled with CFSE according to the protocol in this booklet. Labeled cells were cultured with no stimulus (red histogram), plate-bound anti-CD3 with soluble anti-CD28 (both 1 µg/ml; purple histogram) or Concanavalin A (5 µg/ml; green histogram). At the indicated time points, cells were stained with fluorochrome-conjugated anti-CD4 and analyzed on a Miltenyi MACSQuant cyometer. FlowJo analysis software was used to gate on CD4+ T cells and generate the overlaid histogram plots.

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. Titrate CFSE to get an optimal staining concentration
Cell death	A. CFSE concentration is too high, resulting in cytotoxicity B. Experimental compound is resulting in cytotoxicity	A. Titrate CFSE to get an optimal staining concentration B. Lower concentration of the experimental compound
Broad peak of CFSE in unstimulated controls	A. Poor mixing of CFSE reagent with cells B. Multiple cell types with different proliferative capacity in the well	A. Mix cells with CFSE immediately and well upon addition B. Label cells with fluorochrome-conjugated antibodies to select specific populations

References

1. 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).
2. 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).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from Cayman Chemical Company.

©07/13/2016, Cayman Chemical Company, Ann Arbor, MI, All rights reserved. Printed in U.S.A.