7-AAD/CFSE Cell-Mediated Cytotoxicity Assay Kit

Item No. 600120

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

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
400201	7-AAD Viability Dye (1,000X)	2 vials/50 µl	4°C
600121	CFSE Stock Solution	1 vial/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. Effector and target cells and the appropriate culture medium for culturing them
2. A flow cytometer equipped with a 488 nm excitation laser
3. Bovine serum albumin (BSA)

INTRODUCTION

About This Assay

Cayman's 7-AAD/CFSE Cell-Mediated Cytotoxicity Assay Kit employs CFSE to label target cells within the mixed cell population and 7-AAD to label dead cells. This kit provides an improvement over the traditional ^{51}chromium (^{51}Cr) release assay to assess cell-mediated cytotoxicity. CFSE labeling is more sensitive, does not employ radioisotopes, and cytolysis can be assessed at the single-cell level. The mixed lymphocyte reaction is a cornerstone method of immunology[1], and this kit can help immunologists to detect subtle changes in cytotoxic lymphocyte function. The kit provides sufficient reagents to effectively stain approximately 10^9 cells.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

1. **Cell-Based 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. The diluted buffer is stable at room temperature for one year.

2. **7-AAD Viability Dye (1,000X) Preparation**

   Add 10 µl of Cell-Based Assay 7-AAD Viability Dye (1,000X) (Item No. 400201) to 10 ml of Assay Buffer and mix well.

3. **CFSE Staining Solution Preparation**

   First, prepare a 0.1% BSA/Assay Buffer by adding 10 mg BSA to 10 ml of Assay Buffer. Then make a 1:1000 dilution of CFSE Stock Solution (Item No. 600121) in 0.1% BSA/Assay Buffer and mix well. You will need 1 ml of CFSE Staining Solution per 10^7 cells.

Performing the Assay

NOTES

To properly analyze the data, the following control target cell groups are needed to set up the flow cytometer and compensation:

- Unstained target cells
- Single-stain target cells for each label or stain

The recommended protocol labels cells with CFSE brightly enough to distinguish labeled from unlabeled cells, but dimly enough to not bleed into other channels. However, should brighter staining be desired, BSA can be omitted from the CFSE Staining Solution preparation.

Labeling of Target Cells

1. Obtain target cells for your cytotoxicity assay. Optimal conditions and incubation times for this assay should be determined on an individual basis.
2. Centrifuge the cells at 400 x g for five minutes. Aspirate the supernatant and flick the tube well to break up the pellet.
3. Quickly resuspend cell pellet in CFSE Staining Solution (prepared as described on page 6) at a concentration of 10^7 cells/ml. A uniform suspension should be reached as quickly as possible, as CFSE is taken up almost immediately and local variations in CFSE concentrations can affect staining uniformity. Control target cells (target cells without CFSE) should be resuspended in 0.1% BSA/Assay Buffer.
4. Incubate the cells in the CFSE Staining Solution for 15 minutes at 37°C.
5. Add at least 10 volumes of culture medium containing FBS. Centrifuge the target cells at 400 x g for five minutes.
6. Aspirate the supernatant.
7. Resuspend the target cells in 10 ml of culture medium.
8. Centrifuge the target cells at 400 x g for five minutes.
9. Aspirate the supernatant.
10. Resuspend the target cells in culture medium at a concentration of 10^5 cells/ml.
11. Incubate the cells at 37°C for 30 minutes or longer (but not long enough for the cells to proliferate) in a CO_2 incubator.

Assay Procedure

1. Collect effector cells into tubes. Centrifuge the cells at 400 x g for five minutes to pellet.
2. Resuspend the cells in culture medium at a concentration of 5 x 10^6 cells/ml.
3. Add effector cells to the CFSE-labeled target cell suspension at a predetermined effector/target cell ratio. Some examples are shown in the table on page 8:

Effector:Target Ratio	Effector Cell Suspension	Target Cell Suspension	Target Cell Medium	Final Volume
0	0 ml	1.5 ml	0 ml	1.5 ml
6.25:1	0.125 ml	1 ml	0.375 ml	1.5 ml
12.5:1	0.25 ml	1 ml	0.25 ml	1.5 ml
25:1	0.5 ml	1 ml	0 ml	1.5 ml

Table 1. Addition of effector cells to target cell suspension

4. Incubate the cell mixture for four hours or for a period of time according to your optimal protocol, allowing enough time for cytolytic activity to progress.
5. To stain in a 96 well v-bottom plate as described here, transfer cells into the plate and centrifuge at 400 x g for five minutes. (For staining in tubes, scale volumes up 5-fold).
6. Aspirate the supernatant. *NOTE: If additional surface markers are to be assayed, staining can be inserted at this point in the protocol.*
7. Resuspend the cells in 100 µl of 7-AAD Viability Dye (1,000X) (prepared as described on page 6) and mix well. The control target cells (target cells without CFSE or 7-AAD viability dye or target cells with CFSE staining only) should be resuspended in 100 µl of Assay Buffer.
8. Incubate the cells for 15 minutes in the dark at 4°C.
9. Centrifuge at 400 x g for five minutes and aspirate the supernatant.
10. Resuspend the cells in 200-500 µl of Assay Buffer.
11. The cells are now ready for analysis with a flow cytometer and should be analyzed immediately. Gate on $CFSE^+$ target cells (ex/em 488/525), and then visualize the live/dead cell percentages by 7-AAD exclusion (ex/em 488/647).

ANALYSIS

Performance Characteristics

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

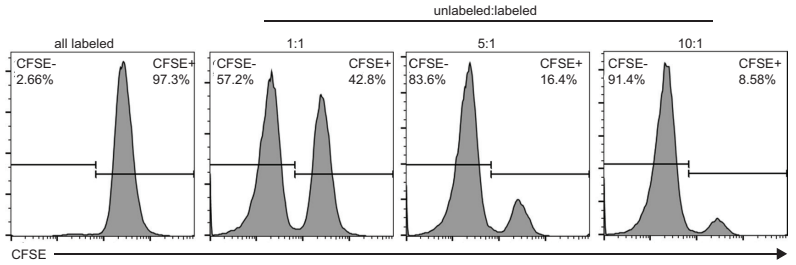

Figure 1: CFSE labels Jurkats for easy detection of minority populations. Jurkat cells were labeled with CFSE according to the protocol, using 0.1% BSA staining buffer. After washing, these cells were mixed with unlabeled Jurkats at the indicated ratios. After 4 hours, CFSE fluorescence was assessed by flow cytometry.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Low signal of CFSE	A. Cells are not healthy B. Cells were not well labeled by CFSE	A. Use only healthy cells B. Perform a titration of CFSE to get an optimal concentration of CFSE staining
No difference in cytotoxicity among different effector cell to target cell ratios	A. Target cells are not healthy B. Effector cells do not cause cytotoxicity	A. Use only healthy target cells B. Use effector cells that cause cytotoxicity

Reference

1. Russell, J.H. and Ley, T.J. Lymphocyte-mediated cytotoxicity. *Annu. Rev. Immunol.* **20**, 323-370 (2002).

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.