CREATIVE BIOMART®
Assay Kit

7-AAD/CFSE Cell-Mediated Cytotoxicity Assay Kit

Product Information

Common Name

7-AAD/CFSE

Cat.No. Kit-1919

Description

The 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 51chromium (51Cr) 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 immunology1, 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.

Kit Components

7-AAD Viability Dye (1,000X), 2 vials/50 µl, 4°C
CFSE Stock Solution, 1 vial/100 µl, -20°C
Cell-Based Assay Buffer Tablet, 4 Tablets, RT

Materials Required 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)

Technical 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

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staining be desired, BSA can be omitted from the CFSE Staining Solution preparation.

Preparation

1. Cell-Based Assay Buffer Preparation
Dissolve each Cell-Based Assay Buffer Tablet 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) 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 in 0.1% BSA/Assay Buffer and mix well. You will need 1 ml of CFSE Staining Solution per 10^7 cells.

Assay Protocol

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 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.

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11. Incubate the cells at 37°C for 30 minutes or longer (but not long enough for the cells to proliferate) in a CO2 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:

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) 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

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7-AAD exclusion (ex/em 488/647).

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