Store at -20°C, 4°C & RT

#72782

7-AAD/CFSE Cell-Mediated Cytotoxicity Assay Kit

1 Kit
(100 assays)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

rev. 08/18/20

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Item #	Quantity	Storage Temp.
7-AAD Viability Dye	89587	2 x 50 µl	4°C
CFSE Stock Solution	16650	1 x 100 µl	-20°C
Cell-Based Assay Buffer Tablet	27868	1 x 4 each	RT

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

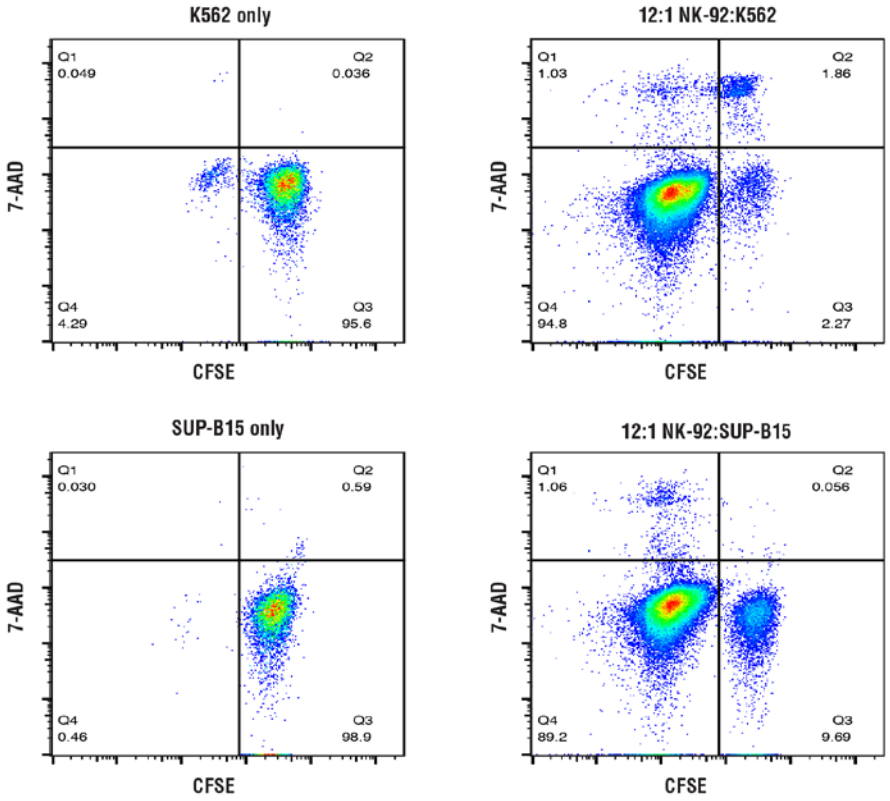
Description: The 7-AAD/CFSE Cell-Mediated Cytotoxicity Assay Kit employs CFSE (carboxyfluorescein succinimidyl ester) to label target cells within the mixed cell population and 7-AAD (7-aminoactinomycin D) to label dead cells. This kit provides an improvement over the traditional chromium-51 (^{51}Cr) 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 using flow cytometry.

Background: Cytolytic assays are routinely used to interrogate immune effector cell function. In order to interrogate immune effector cell cytolytic activity in a heterogeneous cell population of effector and target cells, it is imperative to be able to discriminate between effector and target cell populations with distinct phenotypes. As such, this kit is designed to label live target cells with the membrane permeable fluorescent dye CFSE prior to coculture with unlabelled effector cells, thus permitting a clear separation between live effector and target cells. After incubation of live effector and target cells, the DNA intercalating dye 7-AAD is added to label dying target cells with compromised plasma membranes. By using dyes with distinct spectral properties, a clear separation between four cell populations can be obtained using flow cytometry: live target cells, dead target cells, live effector cells, and dead effector cells (1).

Storage: Kit will arrive packaged as a -20°C kit. For best results, remove 7-AAD Viability Dye and Cell-Based Assay Buffer Tablet and store at 4°C and RT, respectively. CFSE Stock Solution should be stored at -20°C. Product is stable for at least 12 months from date of receipt when stored as recommended.

Background References:

(1) Kim, G.G. et al. (2007) *J Immunol Methods* 325, 51-66.



◀ *Flow cytometric analysis of target K562 cells (top row) or SUP-B15 cells (bottom row) labeled with CFSE, incubated with (right column) or without (left column) effector NK-92 cells at a 12:1 effector:target cell ratio for 4 h, and stained with 7-AAD for 15 min. Cells that stain positive for both 7-AAD and CFSE represent dying target cells and are located in Q2 of the dot plot.*

Thank you for your recent purchase. If you would like to provide a review visit cellsignal.com/comments.

Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#72782

7-AAD/CFSE Cell-Mediated Cytotoxicity Assay Kit

A. Solutions and Reagents

Supplied Reagents:

1. **Cell-Based Assay Buffer:** Dissolve each Cell-Based Assay Buffer Tablet (#27868) in 100 ml of reverse osmosis deionized (RODI) 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:** Add 10 µl of 7-AAD Viability Dye (1,000X) (#89587) to 10 ml of Cell-Based Assay Buffer and mix well.
3. **CFSE Staining Solution:** First, prepare a 0.1% BSA/Assay Buffer by adding 10 mg BSA to 10 ml of Cell-Based Assay Buffer. Then make a 1:500 dilution of CFSE Stock Solution (#16650) in 0.1% BSA/Assay Buffer and mix well. You will need 1 ml of CFSE Staining Solution per 10^7 cells.

   NOTE: This incubation step can be increased to 30 min.

Additional Reagents (Not Supplied):

1. Bovine Serum Albumin (BSA) (#9998)

B. Labeling of Target Cells

NOTE: 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-stained target cells for each label or stain

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 in Solutions and Reagents) 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.

C. 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 below:

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

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 200 µl of 7-AAD Viability Dye (prepared as described in Solutions and Reagents) 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 200 µl of Assay Buffer.
8. Incubate the cells for 15 minutes in the dark at 4°C.
9. 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).

D. Troubleshooting

Problem	Possible Causes
Low signal of CFSE	A. Cells are not healthy B. Cells were not well labeled
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