Cell-Mediated Cytotoxicity Fluorometric Assay Kit (7-AAD/CFSE)

(Catalog # NBP2-54852; Store kit at -20°C)

I. Introduction:

Cell-mediated cytotoxicity is an important phenomenon characterized by cytolysis of a compromised cell in the body by immune system. Activation of immune system leads to removal of target cells infected by pathogens or transformed cells/cancer cells. This process is mediated by antibody-dependent cell-mediated cytotoxicity (ADCC), complement-mediated cytotoxicity, or lymphocyte-mediated cytotoxicity. Novus' Cell-Mediated Cytotoxicity Assay Kit contains carboxyfluorosuccinimide ester (CFSE), a green fluorescent probe that labels live target cells and 7-aminoactinomycin D (7-AAD), a red fluorescent probe that labels late apoptotic and necrotic target cells killed in the cytotoxicity assay. This assay does not require cell lysis and provides a direct measurement of cytotoxicity instead of indirect indicators such as release of ATP or lactate dehydrogenase activity. The flow cytometry-based method provides robust data and enables multi-parametric analysis.

II. Applications:

- Measurement of cell cytotoxicity in response to drug or toxin treatment
- Quantification of the cytotoxic effect of immune effector cells on target cells
- Assessment of physiological mediators and antibodies that affect cell cytotoxicity

III. Sample Type:

- Adherent and suspension cells

IV. Kit Contents:

Components	NBP2-54852	Cap Code
Cytotoxicity Assay Buffer	2x100 ml	NM
7-AAD Staining Solution	0.1 ml	Red
CFSE Staining Solution	0.1 ml	Green

V. User Supplied Reagents and Equipment:

- 6-, 12- or 24-well plate for cell culture
- Flow Cytometer

VI. Storage Conditions and Reagent Preparation:

Kit is shipped at -20°C. Store the reagents according to the specific instructions for each component, protect from light. Centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Cytotoxicity Assay Buffer:** Store at 4°C or -20°C. Warm to 37°C before use. Use within two months.
- **7-AAD Staining Solution:** Store at -20°C. Use within two months.
- **CFSE Staining Solution:** Store at 4°C. Don't freeze thaw. Use within two months.

VII. Cytotoxicity Assay Protocol:

1. Seed cells (10^5-10^6 cells/ml) in an appropriate plate according to the desired protocol. Incubate in a CO_2 Incubator at 37°C for at least 24 hrs before treatment.
2. Treat cells with desired drug, toxin, or effector cells for an appropriate time period (treated cells). Prepare parallel well(s) for untreated cells (vehicle control). After treatment, collect cells in tubes by centrifugation at 1,000 X g for 5 min. at 4°C.
3. Wash cells once with 1 ml of Cytotoxicity Assay Buffer. Resuspend cell pellet in Cytotoxicity Assay Buffer at a concentration of 10^6 cells/ml. Add 1 µl of CFSE Staining Solution and 1 µl of 7-AAD Staining Solution to untreated and treated cells. Incubate for 30 min. at 37°C or longer (but not long enough for the cells to proliferate) in a CO_2 incubator.

   Note:

   a. We recommend staining treated cells with CFSE alone and 7-AAD alone to choose the proper instrument gating set up.

   b. We recommend keeping unstained control cells (i.e. without CFSE or 7-AAD staining) suspended in Cytotoxicity Assay Buffer for both treated and untreated samples to set up the flow cytometer instrument.
4. Analyze immediately using flow cytometry. CFSE cell staining is measured in the FL1 channel and 7-AAD cell staining is measured in the FL3 channel. To ensure that only proper target cells are gated, use a side scatter versus FL-1 plot.

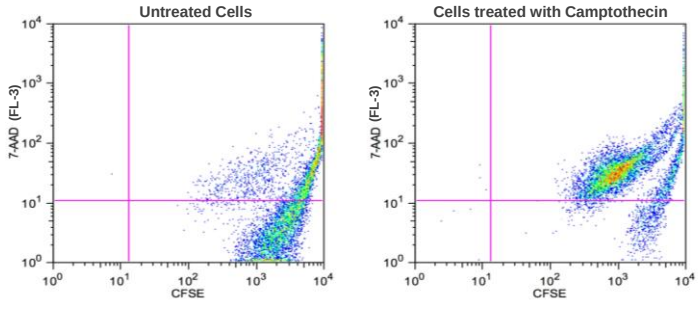

Figure: Cytotoxicity Assay Kit: Jurkat cells (10^5 cells/ml) were grown in RPMI media supplemented with 10% FBS. Cells were treated with camptothecin (5 µM) overnight. Next day, cells were stained with CFSE and 7-AAD for 30 min. at 37°C. The graph (right side) displays the cytotoxic effect of the compound, illustrating apoptosis using CFSE and 7-AAD.