ELISA PRODUCT INFORMATION & MANUAL

Cell-Mediated Cytotoxicity Assay Kit (Fluorometric)

NBP2-54852

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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NBP2-54852 – Cell-Mediated Cytotoxicity Assay Kit (Fluorometric)

For the measurement of cell mediated cytotoxicity in adherent or suspension cells
For research use only - not intended for diagnostic use.

Storage and Stability

The entire kit may be stored at 4°C in the dark for up to 6 months from the date of shipment. Avoid freeze-thaw cycles.

Materials Supplied

Item	Quantity	Storage Condition
Cytotoxicity Assay Buffer	2 x 100 mL	-20°C
7-AAD Staining Solution	0.1 mL	-20°C
CFSE Staining Solution	0.1 mL	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

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.

Assay Procedure

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 CO2 incubator
   Δ Note: *We recommend staining treated cells with CFSE alone and 7-AAD alone to choose the proper instrument gating set up.*

   Δ Note: *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.

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