

Cytotoxicity Assay Kit

Basic Cytotoxicity Test Assay Kit

CATALOG #
AAA52708

PRODUCT SYNONYMS

Cytotoxicity; Basic Cytotoxicity Test Assay Kit; Cytotoxicity assay kit

PRODUCT SPECIFICATIONS

Preparation and Storage

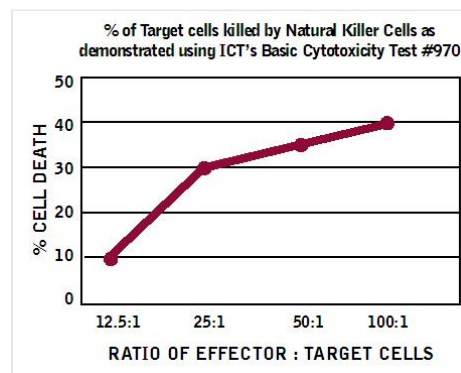
CFSE at -20 degree C
Other Kit Components at 2 - 8 degree C

IMAGES

$$\% \text{ Cytotoxicity} = \frac{\text{Killed Target Cells} = R2}{\text{Killed Target Cells} + \text{Live Target Cells} = R2 + R1} \times 100$$

Application Data

As all target cells were initially stained green, the entire population can be counted in R3 = R2 + R1. After the effector cells and red live/dead stain were added, dead target cells were stained red, shifting them up the Y-axis (R2), clearly distinguishing them from live target cells (R1).



Application Data

ICT's Basic Cytotoxicity Test can be used to easily analyze the activity of natural killer (NK) cells. Based on flow cytometric data, the formula $[R2/(R2+R1)] \times 100$ was used to calculate cytotoxicity. In this example, cytotoxicity increased as more NK effector cells were combined with target cells. As the ratio of Effector:Target-cells increased from 12.5:1 to 100:1, the percentage of target cells dying increased from 10% to 40%.

ADDITIONAL INFORMATION

Related Product Information for Cytotoxicity assay kit	Background/Introduction: Cytolytic activity is an important process for eliminating intracellular pathogens and cancer cells. This process is accomplished through various immune effector mechanisms including natural killer (NK) leukocytes. NK activity is facilitated by non-specifically lysing infected targets through the use of NK receptors, or the Fcγmab1 (CD16) receptor, recognizing IgG bound to specific antigens on the target cell surface¹. NK cells may also induce apoptosis in target cells. The activity of natural killer cells, and their effect on target cells, is frequently studied in immunomodulation experiments. Older methods to assess NK cytolytic activity include measuring the release of lactate dehydrogenase, and more commonly, the release of radioactive ⁵¹Cr from lysed target cells¹. Unfortunately, these techniques have several drawbacks. Traditional enzyme-release assays are often skewed by the large number of necrotic effector cells. Problems associated with ⁵¹Cr release methods include high spontaneous leakage resulting in high backgrounds, high cost, short half-life, and the health risks due to exposure to radioactive material². Flow cytometric assays have been developed to overcome some of the difficulties associated with older assays like lactate dehydrogenase and ⁵¹Cr release assays. One such early version involved the detection of NK cytotoxicity activity by staining target cells with the green fluorescent dye, F-18, in combination with the DNA intercalating dye, propidium iodide³. Since then, a red fluorescent membrane dye, PKH-26, has been used in preference to F-18, and in combination with the viability probe, TO-PRO-3 iodide⁴⁻⁷. However, despite correlations of greater than 95% when compared with the ⁵¹Cr release assay¹, the PKH-26 method is problematic. It is difficult to use at a constant concentration, leading to unreliable staining, and the staining procedure requires multiple steps, often decreasing the viability of the target cells. More recently, the problems with older flow cytometric assays were overcome with the use of 5(6)-carboxyfluorescein diacetate N-succinimidyl ester (CFSE), a green fluorogenic reagent that diffuses into target cells and covalently binds to primary amino groups on intracellular molecules⁸. Intracellular esterases quickly cleave the acetate groups from the dye, thus converting it to its green fluorescent form. Any unbound reagent diffuses back out of the cell. Building upon these techniques, the Basic Cytotoxicity Assay, a flow cytometry assay combining the green fluorescing cellular stain, (CFSE), with a red fluorescing live/dead stain, 7-aminoactinomycin D (7-AAD). The assay can be used to quantify cytolytic killer activity and distinguish target and effector cells, and live and dead cells within a single sample tube. In the Basic Cytotoxicity Assay, CFSE is first used to label the target cell population green (Figure 4). The unstained effector cells are then added and incubated with the target cells (referred to as the Effector:Target, or 'E:T' mixture, Figure 5). As all of the target cells are initially labeled with green fluorescing CFSE, and the effector cells are not, these two populations can easily be differentiated (Figure 7). Upon completion of the E:T incubation, the second reagent, 7-AAD, is added to stain all necrotic cells red by binding to the DNA of membrane-compromised cells (Figure 6). With proper compensation and gating of the flow cytometer using the designated instrument controls (Figures 3 and 7-10), researchers can distinguish between target and effector cells, and living and necrotic cells, and assess the level of cytotoxicity in their samples (Figure 10).
Product Categories/Family for Cytotoxicity assay kit	Apoptosis Assays/Cytotoxicity Testing

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

AAA Biotech | Tel: +1 (619) 604-9440 | Fax: +1 (619) 604-9441 | contact@aaabiotech.com
 Online datasheet: <https://stage.aaabiotech.com/assay-kits/cytotoxicity/52708>