



ACT1

Assay for CytoToxicity

A non-radioactive cytotoxicity assay for flow cytometry

Key Benefits

- No radioactive materials required
- Allows phenotyping of a heterogeneous target cell population
- One-step, no wash assay
- Works with flow cytometer or fluorescence microscope
- Detects cytolytic activity at a cellular level
- Works with multiple types of mammalian cell lines

Introduction

The immune system is able to recognize and destroy target cells, such as tumor or virally/ bacterially (intracellular) infected cells, through cell mediated cytotoxicity (CMC) or antibody-dependent cell-mediated cytotoxicity (ADCC) (1). Evaluation of this CMC/ADCC activity is one of the most important immunoassays to monitor the status of the immune system.

The most commonly used method to measure CMC/ADCC is a radioactive chromium-51 (^{51}Cr) release assays (2). There are several disadvantages with this assay: it is expensive, difficult to load certain cell types, expensive to dispose of due to strict environmental regulations, and has high background from spontaneous release of ^{51}Cr . With the use of flow cytometry, it is now possible to eliminate the need for radioactive material and increase the ability to quantify cytolytic activity on a single cell bases. Various groups have demonstrated that measuring CMC/ADCC activity by flow cytometry has a strong (95%) correlation with the traditional ^{51}Cr release assay (3,4,5,6).

Assay Principle

A cell tracking dye CFSE analog (7,8,9) is utilized to label the target cell population. After the assay has run its experimental protocol, 7AAD (live/dead stain) (10,11) is added to measure cell death. 7AAD only enters membrane compromised cells and binds to DNA.

Flow cytometry is utilized to gate on the target cells and measure 7AAD negative vs. 7AAD positive cells. % cytotoxicity is calculated by the following equation (see experimental example below):

$$\% \text{ Cytotoxicity} = \frac{\text{7AAD positive (upper right quadrant)} - \text{7AAD Positive (upper right quadrant)}}{\text{7AAD Positive (upper right quadrant)} + \text{7AAD negative (lower right quadrant)}} \times 100$$

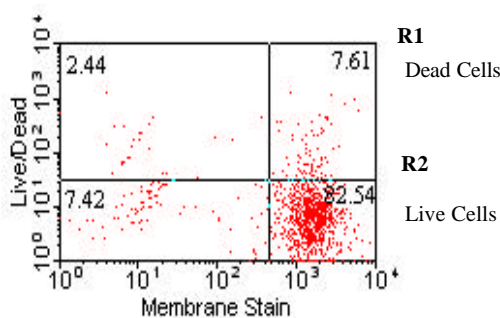


Fig 1

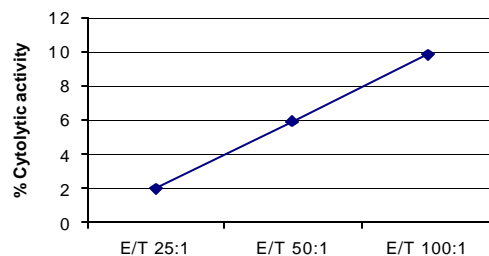


Fig 2

In Fig 1: To test natural killer ability of swine *gd* lymphocytes, K562 cells were stained and adjusted to a final concentration of 1×10^4 cells/100 ul RPMI containing 10 % FBS. *gd* lymphocytes were added at E/T ratios of 25: 1, 50: 1, and 100: 1 and adjusted to a total volume of 400 ul RPMI, then incubated for 4 hours at 37° C in a sterile capped facs tube. Following incubation live/dead stain was added directly to each tube, incubated for 15 min on ice and analyzed by flow cytometry.

In Fig 2: % Cytotoxicity was determined using the formula $(R1/ R1 + R2) \times 100$. The data was plotted in a graph format

Kit Content

- 1) 4 vials of CFSE analog membrane stain, part no 4002.
- 2) 3 vials of 7AAD Live/Dead stain, part no 4003.
- 3) 1 bottle 10X PBS with Ca^{2+} and Mg^{2+} part no 3003

Ordering Information

Catalog #	Size	Price (US\$)
ACT100-2	100 + Tests	\$275

Reference:

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