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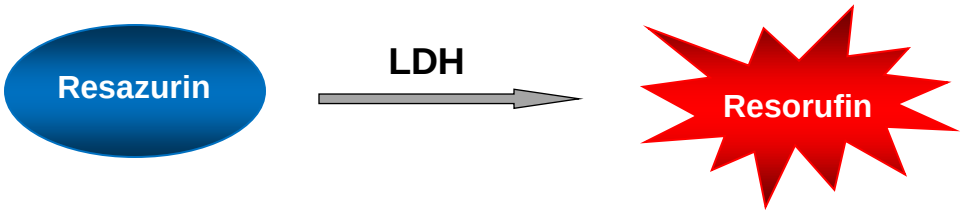
Elite™ Cell Cytotoxicity Assay Kit (Red Fluorescence)

CATALOG NUMBER: CA-C066, 100 assays

Description

The measurement of mitochondrial dehydrogenases (e.g. LDH) activity is a well-accepted assay to quantify cell numbers and monitor cell viability. Elite™ Fluorimetric Cell Cytotoxicity Assay Kit provides a fast, simple, accurate and homogeneous assay for the colorimetric or fluorimetric detection of viable cells. This assay is based on the observation that oxidized non-fluorescent blue resazurin is reduced to a red fluorescent dye (resorufin) by accepting an electron from mitochondrial respiratory chain in live cells. The amount of resorufin produced is directly proportional to the number of living cells.

Elite™ Fluorimetric Cell Cytotoxicity Assay Kit is more sensitive for cell proliferation and cytotoxicity than other assays such as MTT. The kit components are quite stable with minimal cytotoxicity, thus a longer incubation time (such as 24 to 48 hours) is possible if required. The characteristics of its high sensitivity (<100 CHO cells), non-radioactivity and no-wash method make the kit suitable for high throughput screening of cell proliferation or cytotoxicity against a variety of compounds. The assay can be performed in a convenient 96-well and 384-well microtiter-plate format with a filter set of Ex/Em = ~540/590 nm.



Features

- **Continuous:** Easily adapted to automation without mixing or separation
- **Convenient:** Formulated to have minimal hands-on time.
- **Wide Applications:** Cell proliferation and cytotoxicity.
- **Sensitive and Accurate:** As low as 100 cells can be accurately quantified.
- **Non-Radioactive:** No special requirements for waste disposal.

Kit Components

- **Component A:** Assay Solution 1 vial (20 ml)

Storage

Keep in freezer (-20 °C) and avoid exposure to light.

Materials Required (but not supplied)

- 96 or 384-well microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- A fluorescence microplate reader: Capable of detecting emission at 590 nm with excitation at 530-560 nm.

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Assay Protocol (for One 96-Well Plate)

1. **Prepare cells and test compounds:**

   1.1 Plate 100 to 10,000 cells per well in a tissue culture microplate with black wall and clear bottom. Add test compounds into the cells, and incubate for a desired period (such as 24, 48 or 96 hours) in a 37 °C, 5% CO_2 incubator. For blank wells (medium without the cells), add the same amount of compound buffer. The suggested total volume is 100 µl for a well of a 96-well plate, and 50 µl for that of a 384-well plate.

   1.2 Set up the following controls at the same time.

   - **Positive control** contains cells and known proliferation or cytotoxicity inducer.
   - **Negative control** contains cells but no test compounds.
   - **Vehicle control** contains cells and the vehicle used to deliver test compounds.
   - **Non-cell control** contains growth medium without cells.

     Note: LDH in serum will contribute to background fluorescence.
   - **Test compound control** contains the vehicle used to deliver test compounds [Hank's balance salt solution (HBSS) or phosphate-buffered saline (PBS)] and test compound. Some test compounds have strong autofluorescence and may give false positive results.

     Note: Match the total volume of all the controls to 100 µl for a well of a 96-well plate or 50 µl for that of a 384-well plate with growth medium.

2. **Assay procedures:**

   2.1 Thaw and warm up the Assay Solution (**Component A**) to 37 °C, and mix it thoroughly before starting the experiments.

   2.2 Add 20 µl/well (96-well plate) or 10 µl/well (384-well plate) of Assay Solution (Component A). Mix the reagents by shaking the plate gently for 30 seconds.

   2.3 Incubate the cells in a 37 °C, 5% CO_2 incubator for 1 to 24 hours, protected from light.

   Note 1: The appropriate incubation time depends on the metabolism rate of the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

   Note 2: Extremely prolonged incubation time is not recommended since resazurin could be converted to colorless dihydroresorufin.

   2.4 Monitor the fluorescence intensity (bottom read) at Ex/Em = 540/590 nm. Alternatively, read the O.D. at 570 nm (the reference wavelength should be 600 nm) to determine the cell viability in each well.

3. **Perform data analysis:**

   3.1. The background fluorescence reading from the non-cell control well is subtracted from the values for those wells containing the cells.

   Note: The background fluorescence of the blank wells may vary depending on the sources of the growth media or the microtiter plates.

   3.2. The fluorescence reading in each well indicates the cell number in that well.

   3.3. Calculate the percentage of cell viability for samples and controls based on the following formula:

   % Cell viability = $100 \times (F_{sample}-F_o)/(F_{ctrl}-F_o)$

   F_{sample} is the fluorescence reading in the presence of the test compound.

   F_{ctrl} is the fluorescence reading in the absence of the test compound (vehicle control).

   F_o is the averaged background (non-cell control) fluorescence intensity.

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Data Analysis

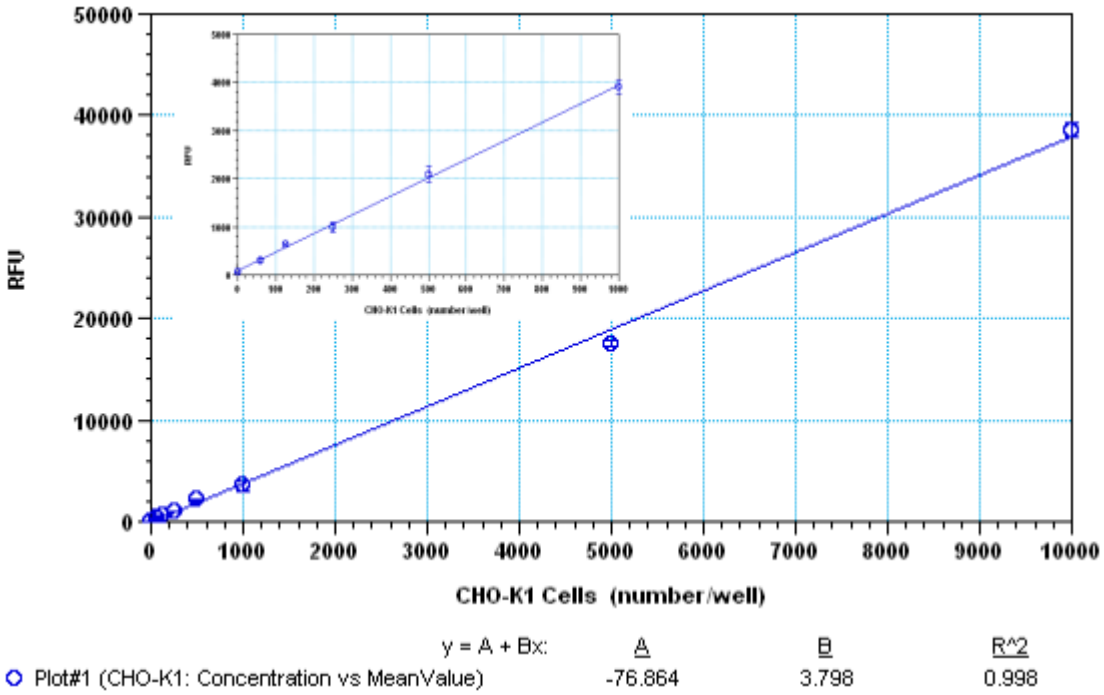

Figure 1. CHO-K1 cell number response was measured with Elite™ Fluorimetric Cell Cytotoxicity Assay Kit. CHO-K1 cells at 0 to 10,000 cells/well/100 µl were seeded overnight in a Costar black wall/clear bottom 96-well plate. The cells were incubated with 20 µl/well of Assay Solution (Component A) for 3 hours at 37 oC. The fluorescence intensity was measured at Ex/Em = 540/590 nm with NOVOstar instrument (BMG Labtech). The fluorescence intensity was linear (R^2 = 0.998) to the cell number as indicated. The detection limit was 60 cells/well (n=6). The insert shows the enlargement of the lower end of the cell number response.

References

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