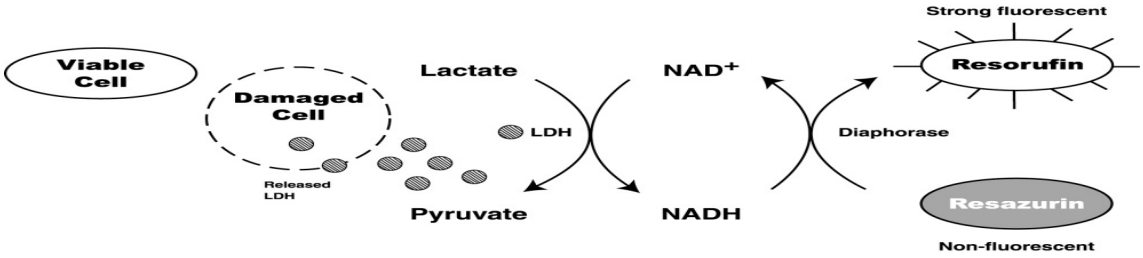
Cell Viability/Cytotoxicity Detection Kit (LDH based)

(Cat# K025-500; fluorometric assay; store at -20°C)

Introduction

Lactate dehydrogenase (LDH) generally exists in prokaryotic, fungal and eukaryotic cells. The measurement of cytoplasmic LDH activity is a well-accepted assay to quantify viable cell numbers and monitor cell proliferation. On the other hand, the leakage of cytoplasmic LDH caused by the damage of cell membrane integrity is also a good indicator of cell death and is used to estimate cytotoxicity.

ABS_Bio™ LDH based Cell Viability/Cytotoxicity Detection Kit provides the easiest and sensitive means for cell proliferation and cytotoxicity assays, utilize resazurin to measure LDH activity either in the cytoplasm for live cells or in the medium for dead cells. The entire assay can be performed in a microtitler plate in mix-read format, measuring the fluorescence intensity at Ex/Em=530/590 nm. The reagent is no toxicity for cells, after the measurement of cell viability; the cells can be used for further experiments. The kit is supplied with enough reagents for 500 tests in 96-well plate and 1000 tests in 384-well plate.



Kit Components

Substrates: Powder Assay Buffer: 30 mL Lysis Buffer: 5 mL Dye: 5 mL

Storage and Handling: The kit is shipped on ice. Store all of the components at -20°C. Shelf Life: 6 months after receipt.

Applications

Cytotoxicity and Proliferation assay: adherent and non-adherent cells, and certain tissues. Detect proliferation in bacteria, yeast, fungi and protozoa as well.

Protocol

Cell Viability Procedure

1. Seeds 1x 10^{2-5} cells in black 96-well (clear bottom) cell culture plate in 100 µL of medium with or without the test compounds. Perform triplicate, no-cell but with media and compounds as blank control.
2. Incubate cells for desired time in standard culture condition.
3. Equilibrate the Dye reagent to room temperature, add 10 µL of the dye reagent to each well, and gently mix the plate.
4. Continue culture 1-3 hours.
5. Measure fluorescent intensity at Ex/Em=530-560/590 nm and determine the cell viability.

$$\text{Cell Viability (\%)} = \frac{F_{sample} - F_{blank}}{F_{cell\ only} - F_{blank}} \times 100$$

F_{sample} are cells with test compounds; $F_{cell\ only}$ are cells without test compounds; F_{blank} are blank control average fluorescence intensity.

Note: LDH contained in serum will contribute to background fluorescence.

Cell Cytotoxicity/Cell Death Procedure

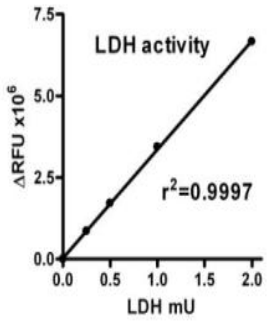
1. Seeds 1x 10^{2-5} cells in black 96-well (clear bottom) cell culture plate in 100 µL of medium with or without the test compounds. Perform duplicate experiments, no-cell but with media and compounds as blank control. **All experiments must have parallel wells for Lysis assay.**
2. Incubate cells for desired time in standard culture condition.
3. Equilibrate all of the components to room temperature, add 22 mL assay buffer into substrate bottle to reconstitute substrate mixture. Mix the substrate mixture completely. Store unused portion of substrate mixture at -20°C.
4. Add 10 µL of PBS to half of the parallel wells of samples and controls; as while add 10 µL lysis buffer to rest half of the parallel wells of samples and controls, and gently shake the plate for 1-2 min on a shaker.
5. Prepare enough working solution for each reaction well by mix 40 µL substrate mixture and 10 µL dye.
6. Add 50 µL of working solution per well and incubate at room temperature for 10 minutes.
7. Measure fluorescent intensity at Ex/Em=530-560/590 nm and determine the cytotoxicity.

$$\text{Cell Cytotoxicity/Cell Death (\%)} = \frac{\Delta F_{\text{Unlyzed cells}}}{\Delta F_{\text{lyzed cells}}} \times 100$$

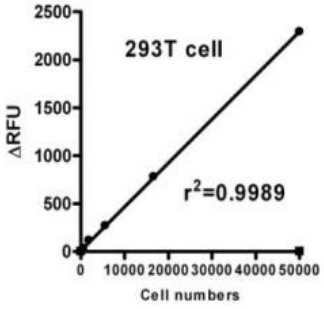
$\Delta F_{\text{Unlyzed cells}}$ are unlyzed cell (F_{sample} - F_{blank}); $\Delta F_{\text{Lyzed Cell}}$ are lyzed cell (F_{sample} - F_{blank}); Fsample are cells with test compounds; F_{blank} are no-cell blank control average fluorescence intensity.

Note: LDH contained in serum will contribute to background fluorescence.

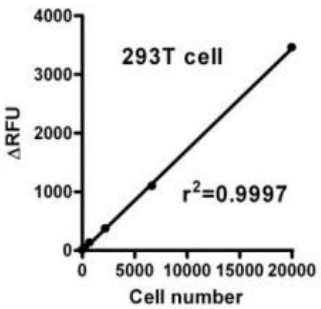
Typical Standard Curve



LDH activity in 96-well plate assay



293T cell Proliferation assay in 96-well plate



293T cell Cytotoxicity assay in 96-well plate

References

Weidmann E. et al. (1995) Ann Hematol 70: 153-158.
Kawai et al. (1992) Cancer Immunol Immunother 35: 225-229
Korzeniewski C. (1983) J Immunol Methods 64: 313-320

Related Products:

Trypan Blue dye (#C8039)

Cell Viability/Cytotoxicity Detection Kit(WST-8 based; #K030-500)

Cell Viability/Cytotoxicity Detection Kit(WST-1 based; #K010-500)

Scratch Wound Healing Assay Kit (#K040-100)

Janus Green Cell Stain Solution (#C8070)

Crystal Violet Stain Solution (#C8072)