SensoLyte® Cell Viability and Proliferation Assay Kit *Fluorimetric*

Revision number: 1.2	*Last updated: October 2014*
Catalog #	**AS-71300**
Kit Size	2,000 Assays (96-well plate)

- ***Convenient Format:*** One solution and one step for the whole assay.
- ***Optimized Performance:*** Optimal conditions for the detection of cell proliferation.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Assay solution (20 mL x 2 bottles)	(20 mL x 2 bottles)

Other Materials Required (but not provided)

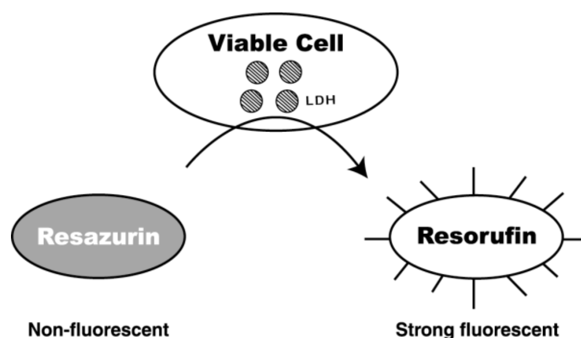
- 96-well microplate: Black tissue culture microplate with or without clear bottom.
- Fluorescence microplate reader: Capable of detecting emission at 590 nm with excitation at 530-560 nm.

Storage and Handling

- Store the entire kit at -20°C, and keep away from light

Introduction

Dehydrogenases exist ubiquitously in mammalian cells and the measurement of mitochondrial dehydrogenases activity is a well-accepted assay for measuring cell numbers and indicating cell viability.[1,2] The SensoLyte® Cell Viability and Proliferation Assay Kit provides researchers with a convenient one-step assay for measuring dehydrogenase activity in live cells. Cell proliferation can be continuously monitored over time (Figure 1), with resazurin used as a sensitive redox indicator.[3-5] Resazurin is reduced to the strongly fluorescent resorufin by accepting electrons from mitochondrial respiratory chain in live cells (Scheme 1). The kit can detect as few as 48 cells (Figure 2), and is suitable for high throughput screening of cell proliferation or cytotoxicity effect of a variety of compounds.



Scheme 1. The dehydrogenases (e.g. LDH) in the living cells will continuously reduce resazurin to the strongly fluorescent resorufin.

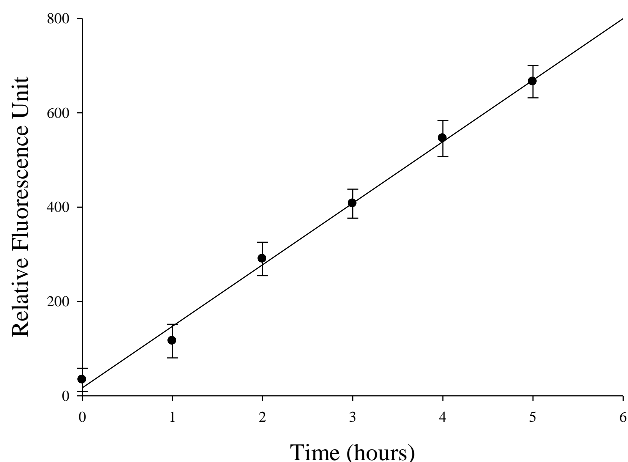


Figure 1. Monitor cell proliferation continuously by monitoring the change in fluorescence.
$1X10^4$ Jurkat cells were seeded into a 96-well plate. 20 µL off assay solution was added and incubated with cells at 37°C. Fluorescence was monitored at Ex/Em=530±30/590±30 nm up to 5 h. (Mean ±S.D., n=four independent samples).

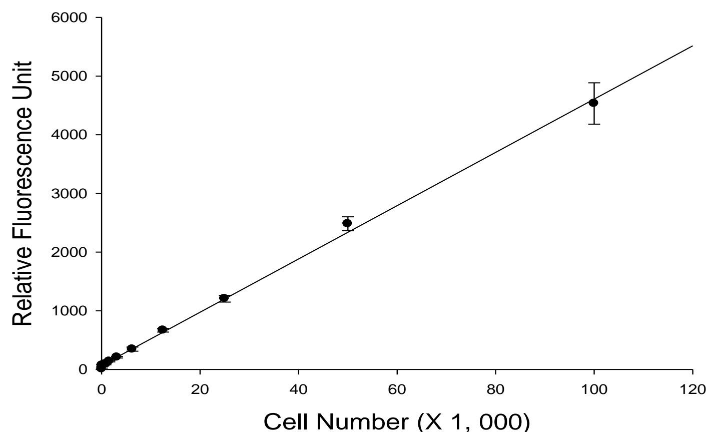


Figure 2. An increase of fluorescent signal is correlated with an increase of cell number. Different amounts of Jurkat cells were seeded into a 96-well plate. 20 µL of assay solution was added and incubated with cells for 3 h at 37°C. Fluorescent signal was monitored at Ex/Em=530±30/590±30 nm. The assay can detect as few as 50 cells (>±3S.D.), with a linear range of up to $1X10^5$cells (r^2=0.99). (mean±S.D., n=four independent samples).

Protocol

1. Prepare cell culture.

1.1 Seed $1X10^{2-5}$ cells per well in a microplate. After adding the test compounds, culture cells in a 37°C incubator for the desired time period. The recommended total volume is 100 µL (96-well plate).

1.2 Simultaneously set up the following controls as deemed necessary:

- 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.
- No-cell control contains growth medium but no cells.

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

- Test compound control contains growth medium and test compound. Some test compounds have strong auto fluorescence and may give false results.

Note: Bring the total volume of all the controls to 100 µL (96-well plate) using growth medium.

2. Perform the assay.

2.1 Warm assay solution (Component A) in a 37°C water bath until thawed. Mix the assay solution completely before use.

2.2 Add 20 µL/well of assay solution (Component A). Mix the reagents by shaking the plate gently for 30 sec.

2.3 Incubate cells at 37°C for 2 to 24 h, keep away from light.

Note: The incubation time is dependent on the metabolic rate of the particular cell line. Cells with faster metabolic rate need shorter incubation time, while cells with slower metabolic rate need longer incubation time. Prolonged incubation time, however, is not recommended, since resazurin will be further converted to colorless dihydroresorufin.[3]

2.4 Measure fluorescence at the emission wavelength of 590 nm with excitation wavelength at 530-560 nm.

3. Data analysis:

3.1 The fluorescence reading from the no-cell control well is the background fluorescence. The readings from other wells need to be subtracted with this background fluorescence.

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

References

1. Mosmann, T. *J. Immunol. Methods* **65**, 55 (1983).
2. Roehm, NW. et al. *J. Immunol. Methods* **142**, 257 (1991).
3. DeBaun, RM. and G. de Stevens, *Arch. Biochem.* **31**, 300 (1951).
4. De Jong, DW. and WG. Woodlief, *Biochim. Biophys. Acta* **484**, 249 (1977).
5. Korzeniewski, C. and DM. Callewaert, *J. Immunol. Methods* **64**, 313 (1983).