APPLICATION NOTE

Quantifying Cell Viability with Resazurin Assay

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Application Benefits

- One-step homogeneous workflow.
- Non-destructive cell viability assay.
- High sensitivity and broad dynamic range.
- Compatible with 96- and 384-well microplates.
- Ideal for drug screening and IC_{50} determination.
- Excellent reproducibility.

Key words
resazurin, resorufin, cytotoxicity, viability, fluorescence

Introduction

Resazurin assay has been used, since 1939, for detecting bacterial contamination in milk and assessing antifungal susceptibility. After that the assay has been applied to evaluate motile sperm density, viability of mammalian cell lines, primary cells, and three-dimensional tumor spheroids[1,2].

Resazurin Viability Assay, also known as AlamarBlue® assay[3], is based on the conversion of resazurin, a blue, non-fluorescent redox indicator, into resorufin, a pink, highly fluorescent compound (*Fig. 1*).

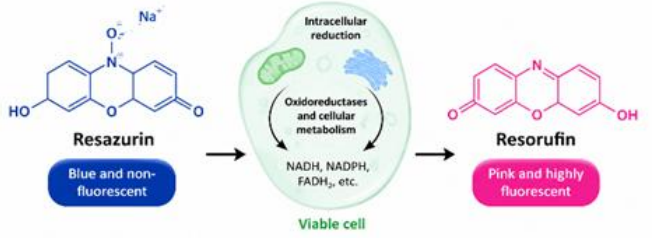

Figure 1. Resazurin reaction. *Resazurin is a low-molecular-weight phenoxazin-3-one dye that is converted to resorufin by NADH, NADPH, FADH, and other endogenous reducing species in reactions catalyzed by mitochondrial or cytoplasmic reductases.*

After entering viable cells, resazurin accepts electrons generated by normal cellular metabolism through the action of multiple intracellular oxidoreductases. The accumulation of resorufin is proportional to the metabolically active cells and, within an appropriate linear range, correlates with the number of viable cells.

Since the readout reflects cellular metabolic activity rather than cell number directly, experimental conditions should be optimized for each cell type, culture conditions and treatment, particularly when compounds are expected to alter cellular metabolism independently of cell proliferation or viability.

Because resazurin reagent is non-destructive and exhibits low cytotoxicity, it enables both endpoint and kinetic measurements in homogenous format without the need for cell lysis. The assay can be quantified by either absorbance at 570 nm or fluorescence at 530-570 nm excitation/ 590 nm emission, making it compatible with a wider range of laboratory instrumentation. However, greater sensitivity is achieved using the fluorescent readout.

Although the assay is highly sensitive, the dynamic range depends on the metabolic activity of the cell type, assay format and incubation time. Therefore, this parameters should be optimized[4].

Optimization Workflow

Cell Culture

Human cervical adenocarcinoma HeLa cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA). It was cultured in DMEM High Glucose medium at 37 °C, supplemented with 10% FBS, 2 mM glutamine, 100 U/mL of penicillin, 100 µg/mL of streptomycin.

Cell Assay

Resazurin Viability Assay Kit (Tiaris Biosciences, Spain) was used. All experiments were performed in triplicate. Fluorescence was measured at wavelengths of 540 nm excitation and 590 nm emission. Data are presented as mean ± standard desviation.

Dynamic range of resazurin assay

HeLa cell suspension was diluted in the growth medium. 100 µL of cells were plated in 96-well plates and grown at 5% CO_2 at 37º C for 24 h. After growth medium was removed, the cells were rinsed with PBS and resazurin solution was added. Different times of resazurin incubation were analyzed.

Cytotoxicity evaluation

HeLa cells (5 × 10^3 cells/well) were seeded in a 96-well plate overnight. Next day, cells were treated with different concentrations of doxorubicin. After 48 h of treatment, cell viability was assayed by using resazurin assay and incubated for 60-90 minutes under the same culture conditions. Cell viability was calculated using the formula: $\%\ Viability = \frac{RFU_{CONTROL} - RFU_{BLANK}}{RFU_{SAMPLE} - RFU_{BLANK}} \times 100.$

Results and Discussion

Dynamic Range Dependence on Number of Cells Seeding and Assay Time.

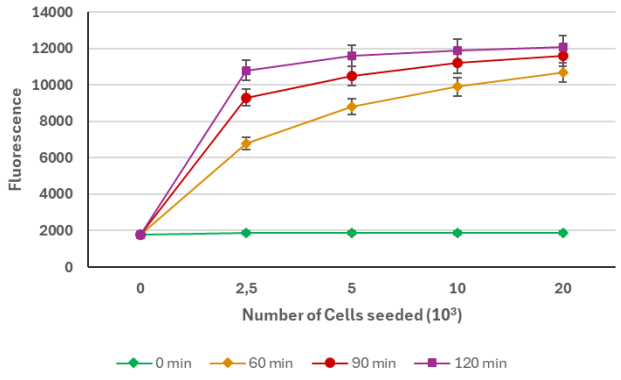

Figure 2. Effect of cell seeding density and resazurin incubation time. *Resazurin reagent was added and fluorescence was measured at different incubation times.*

To evaluate the performance of the resazurin assay, fluorescence was measured at different cell seeding densities after incubation with the reagent (*Fig. 2*).

At low to moderate seeding densities (2,5–5x10^3 cells/well), the fluorescence signal increased proportionally with cell number, indicating a linear assay response suitable for quantitative measurements of cell viability.

At higher cell densities (>10000 cells/well), the rate of signal increase progressively decreased suggesting assay saturation. Under these conditions, additional increases in cell number resulted in only modest increases in fluorescence, reducing the dynamic range of the assay.

Extending the incubation time from 1 to 2 hours substantially improved signal intensity while maintaining good discrimination between different cell densities.

These results demonstrate that both cell seeding density and incubation time are critical parameters influencing assay performance.

Table 1. Recommended assay conditions for HeLa cells

Parameter	Recommended Condition
Cell Seeding Density	2,5-5 x 10^3 cells/ well
Plate Format	96-well plate microplate
Resazurin Incubation	60-120 min
Detection	Fluorescence
Assay Response	Linear under the recommended conditions

Doxorubicin treatment of HeLa Cell Line

Doxorubicin is one of the most widely used positive control compounds for *in vitro* cytotoxicity assays. Its consistent cytotoxic activity in a wide range of cell lines and well-characterized mechanism of action makes it an ideal reference compound for assay optimization, performance validation, and determination of dose–response parameters such as the IC_{50} in resazurin-based cell viability assays.

Treatment with doxorubicin shows that cell viability progressively decreased as the concentration of doxorubicin increased from 0 to 100 nM, demonstrating a clear concentration-dependent cytotoxic effect (*Fig. 3*).

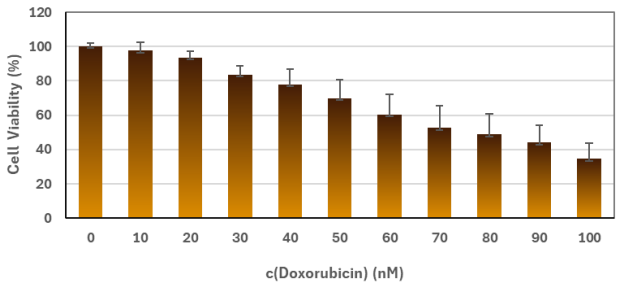

Figure 3. Doxorubicin treatment of HeLa cells. *Cells were treated with different concentrations of doxorubicin for 48 h before addition of Resazurin reagent.*

Only a slight reduction in viability was observed at low concentrations (10–20 nM), indicating limited cytotoxicity within this range. From approximately 30–50 nM onwards, the decrease in cell viability became more pronounced, suggesting the onset of a stronger biological response.

Overall, these results confirm the expected cytotoxic activity of doxorubicin and demonstrate that the resazurin assay provides a sensitive and reproducible method for quantifying concentration-dependent changes in cell viability.

Conclusion

- Resazurin approach as a redox-based metabolic assay, provides a rapid and convenient means of estimating the number of viable cells by exploiting the ability of metabolically active cells to reduce cell-permeable oxidation-reduction indicator resazurin into a detectable product.
- The assay exhibited a linear response at low to moderate cell densities, whereas prolonged incubation and high cell densities resulted in progressive signal saturation.
- The Tiaris Resazurin Viability Assay Kit provides a rapid, sensitive and reproducible solution for quantitative cell viability analysis and drug-response profiling in 96- and 384-well formats.

References

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