MEASURING CELL-VIABILITY BY RESAZURIN (ALAMARBLUE®) ASSAY USING PHOTOPETTE® CELL

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- *Photopette Cell makes the measurement of cell viability and proliferation easy and efficient.*
- *Using alamarBlue® (Resazurin) as a REDOX indicator, the innate metabolic activity of cells could be quantified by Photopette Cell via time-course monitoring measurements.*

OBJECTIVE

The objective of this application note is to demonstrate how Photopette Cell can be used to measure cell-viability of eukaryotic cells using a Resazurin Assay. Furthermore, it acts as a handy guide to get you started with Photopette Cell, and outlines application-specific parameters for reference.

INTRODUCTION

Cell-viability assays are important cell-based assays to determine whether cells are alive or dead. Such assays are widely used for investigating cell response to a given agent or drug or to establish relative cytotoxicity of chemicals. The cell-viability is typically measured by determining innate metabolic activity using redox indicators.

Resazurin dye is used in redox based colorimetric assays to determine cellular metabolic reduction with minimal cell-toxicity. Living cells maintain a reducing environment within their cytoplasm and mitochondria. This reducing environment of living cells causes the Resazurin indicator to change from the oxidized-form (blue) to the reduced form-(red). By monitoring the absorbance at 570 nm and 600 nm, relative metabolic activity for the cells can be determined. alamarBlue® is an assay based on the Resazurin dye. The absorbance spectrum for reduced and oxidized forms of the Resazurin dye are highlighted in Figure 1.

MATERIALS AND APPARATUS

Instruments:

- Photopette® Cell

Reagents:

- Resazurin sodium salt (Sigma-Aldrich #R7017)
- PBS 10x (Sigma-Aldrich #P5493)
- YPD Broth (Sigma-Aldrich #Y1375)
- Yeast (Sigma-Aldrich #YSC2)
- De-ionized water

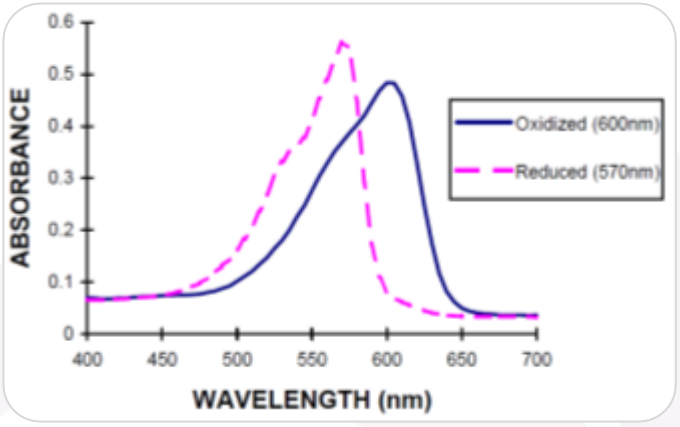

Fig 1: Absorbance spectrum of the oxidized and reduced form of alamarBlue® (Resazurin) [1]

METHOD

The highlighted protocol is based on the alamarBlue® assay protocol [1]. It is advised to perform an application specific risk-assessment analysis before performing experiments. Please refer to the Photopette User Manual for operating and safety precautions [2].

EXPERIMENTAL PROCEDURE

Preparation

Prepare YPD media for yeast culture as per the manufacturer's instruction and autoclave it to ensure it is sterile [3]. Prepare 1x PBS solution by 1:10 dilution with de-ionized water. Prepare Resazurin stock solution (100x) by dissolving 0.50 g Resazurin sodium salt into 100 ml 1x PBS.

Prepare working solution for Resazurin by 1:100 dilution of the prepared stock solution using 1x PBS.

Prepare yeast culture by adding yeast to the prepared YPD media and incubate the culture overnight at 37 °C under shaking. Subsequently, if needed, determine the yeast cell-count by using a hemocytometer. Dilute the yeast culture with sterile YPD media to about 60,000 cells per ml and store the cells in an ice bath.

Experimental

Turn-on the Photopette® device and connect to the Photopette® iOS/Android app. Select the application 'Resazurin/alamarBlue' under measurement type. Select dataset and set additional settings (if needed) before selecting *'Start Measurement'*. Please follow the video-tutorials available at www.tipbiosystems.com in order to get familiar with the measurement process.

Use sterile YPD media for the blank measurement. Place a CuveTip™ firmly onto the device probe and insert it into the blank sample to perform the auto-zero measurement. Ensure that there is no air-bubble trapped in the CuveTip™ cavity. Presence of air bubbles can disrupt the optical path and may cause errors.

Bring the diluted yeast culture of 60,000 cells/ml to room temperature and resuspend cells well. Transfer 1.5 ml of the diluted yeast culture into a 2 ml centrifuge tube; prepare several such aliquots depending on the number of desired repeats. Subsequently, add 150 µl of the Resazurin working solution to the aliquots. Start taking measurements every few minutes. The cell-type and metabolic activity will influence the sampling period. For example, sample every 15 minutes for a period of 3 hours. For each sampling, insert the Photopette® device with its attached CuveTip™ into the sampling solution and perform a measurement. Keep the yeast cultures (with added Resazurin) in a dark-environment at room temperature.

DETERMING CONCENTRATION OF AN UNKNOWN SAMPLE

Following a similar approach, any eukaryotic cell-culture can be measured for cell viability by measuring absorbance at 570 nm and 600 nm using Photopette® Cell. A higher reduction rate indicates a higher number of cells or more active cells.

DISCUSSIONS

EXPERIMENTAL PARAMETERS

Time-course analysis

Cells' innate metabolic can be measured by using an established formula for reduction percentage. Reduction percentage can be calculated by following formula [1] :

$$\text{Reduction Percentage} = \frac{\varepsilon_{OX\_600nm} \times A_{570nm\_tx} - \varepsilon_{OX\_570nm} \times A_{600nm\_tx}}{\varepsilon_{RED\_570nm} \times A_{600nm\_t0} - \varepsilon_{RED\_600nm} \times A_{570nm\_t0}}$$

where, ε is the Molar Ext. Coefficients for Resazurin,
A is the measured absorbance at a given wavelength,
t_0 is the first measurement, and
t_x is the measurement at a given time x.

The molar extinction coefficients for the oxidized and reduced forms of alamarBlue® is provided in Table 1 below.

Wavelength	Reduced Resazurin ε_{RED}	Oxidized Resazurin ε_{OX}
570 nm	155,677	80,586
600 nm	14,652	117,216

Table 1: Molar Extinction Coefficients for alamarBlue® [1]

The absorbance values for 570 nm and 600 nm wavelengths thus obtained are tabulated in Table 2. The reduction percentage was calculated using the above equation.

Time *(mins)*	Absorbance 570 nm	Absorbance 600 nm	Reduction Percentage
0	0.79	0.97	11
14	0.83	0.92	17
30	0.86	0.89	21
46	0.88	0.86	24
60	0.89	0.84	26
74	0.91	0.82	29
90	0.92	0.80	31
106	0.93	0.78	33
120	0.94	0.77	35
134	0.95	0.76	36
150	0.96	0.74	38
180	0.98	0.71	41

Table 2: Absorbance values for 570 nm and 600 nm, along with calculated reduction percentage

The corresponding plot of the data from Table 2 is highlighted in Figure 2 below.

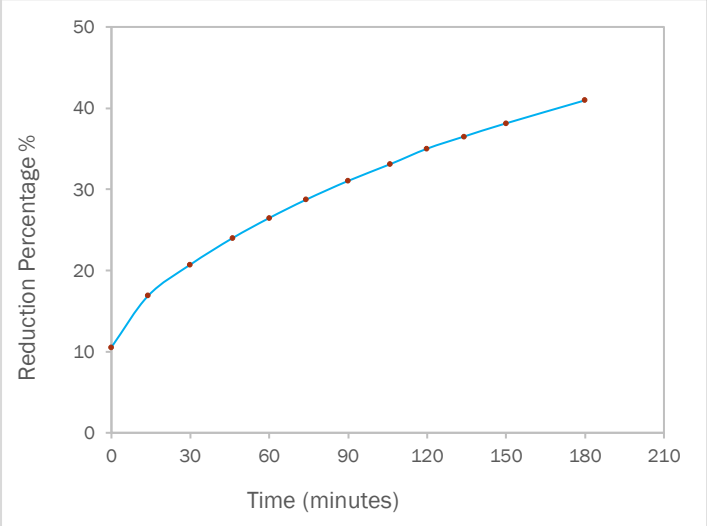

Fig 2: Relative metabolic activity of yeast cells measured by the percentage of the reduced form of Resazurin.

To perform a cytotoxicity experiment, a second set of yeast samples with various concentrations of a toxin, chemical or drug must be prepared. The difference in the Reduction Percentage between the reference cells (no toxin added) and the samples with added toxin is proportional to the toxicity of the added toxin, chemical or drug.

LIMITATIONS

As highlighted in the alamarBlue® protocol notes, the starting conversion may not be zero. However, this can be easily handled by subtracting the initial reduction percentage values with the reduction percentage at a given time.

SUMMARY

The application note guides in determining cell-viability using a Resazurin based assay and Photopette® Cell.

REFERENCES

[1] "alamarBlue® Assay."

[2] Tip Biosystems Pte Ltd, F. Omar, "Photopette User Manual V1.0.0," Singapore, 2016.

[3] "YPD Broth for microbiology | Sigma-Aldrich." [Online]. Available: http://www.sigmaaldrich.com/catalog/product/sigma/y1375. [Accessed: 29-Mar-2017].