The Use of Baker’s Yeast in the Resazurin Reduction Test: A Simple, Low-Cost Method for Determining Cell Viability in Proliferation and Cytotoxicity Assays†

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INTRODUCTION

Conducting cell viability experiments as a laboratory activity in a class is sometimes not possible, especially in an institution where the budget is tight. Typically, cell viability experiments require very expensive testing kits that are limited to a few assays and test cells that need to be maintained in expensive culture medium. Furthermore, many institutions have biosafety considerations for the use of certain cell lines in research and instruction activities. I have developed a simple, low-cost laboratory activity for testing the cellular proliferative and cytotoxic effects of certain substances using baker’s yeast (Saccharomyces cerevisiae) in the resazurin reduction test (RRT).

The RRT is based on the ability of living cells to reduce the blue resazurin (7-hydroxy-3H-phenoxazin-3-one 10-oxide) into pink resorufin. Living cells have active N-methyl-phenazinium methosulfate that catalyzes the redox reaction of NADH and blue resazurin to produce pink resorufin. This test is used as an oxidation-reduction indicator in cell viability assays for bacteria and mammalian cells. A comparison of a laboratory-prepared resazurin solution’s activity with a resazurin-based commercial product showed that they produce identical results.

Choosing the treatments

Prior to conducting the activity, students must already be trained on how to use micropipettes and how to properly dispense micro volumes of liquids. They must also know how to properly sterilize laboratory apparatus. Most importantly, they must already be acquainted with the proper safety equipment and techniques to work in a BSL1 laboratory.

PROCEDURE

Required materials

Resazurin solution and yeast suspension are the primary materials needed for this activity. The protocols for the preparation of these materials are detailed in Appendix I and 2, respectively. Other materials needed include sterile 96-well microplates, a micropipette set, sterile pipette tips, sterile beakers, aluminum foil, a digital camera, and a UV-Vis spectrophotometer if available.

Laboratory skills to be covered prior to the activity

Prior to conducting the activity, students must already be trained on how to use micropipettes and how to properly dispense micro volumes of liquids. They must also know how to properly sterilize laboratory apparatus. Most importantly, they must already be acquainted with the proper safety equipment and techniques to work in a BSL1 laboratory.

Choosing the treatments

The instructor can choose or suggest to the class what substance to test (suggested treatments are listed in Appendix 3). However, it is recommended that the students, by group, be allowed to decide what substances they want to use as experimental treatments so that they will be more engaged with the activity. If the students are given the freedom to choose, the instructor should give the students a week’s notice of the activity so they will have time to prepare.

Furthermore, it is also important to choose which control treatments to use in the experiment. While S. cerevisiae cells to be treated with only sterile distilled water would obviously serve as the negative control, two positive controls are also needed. One positive control must already be proven to exhibit cytotoxicity to the cells, and the other must promote proliferation of the S. cerevisiae cells. Suggested control treatments are also listed in Appendix 3.

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Tips & Tools

cheaper classroom laboratory activity that involves testing the effects of some substances on cell viability.

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Designing the experiment

When the principle of the test is already well understood by the students and if all treatments are already identified, let the students design the experiment. This could be done by giving each group a template of a 96-well microplate and allowing them to assign wells for the different treatments and their respective replicates. This template is important in identifying and tracking which well in the microplate has already received a particular treatment, especially if the treatment is colorless.

Cell viability assay

The complete procedure for doing the assay is found in Appendix 4. The key to a successful assay is the result of the controls. If the wells with cells treated with the positive cytotoxic control have remained blue, it means the cells were already dead. Finally, if the wells with cells treated with a positive control for promoting cell proliferation turned pink, it means the cells have multiplied significantly and that they were able to reduce the blue resazurin into pink resorufin.

Conversely, if the wells of cells treated with only sterile distilled water are colored violet, it means that the cells are still alive and that the sterile distilled water does not contain other substances that cause death or proliferation of all the cells. Although within the given incubation period some cells might die because of the absence of a carbon source, these natural deaths are not significant compared with the cell death induced by the positive control.

Gathering and analyzing the data

To compare the effects of the different treatments on the cells, absorbance at 600–630 nm (the peak absorbance of resazurin) or 570 nm (the peak absorbance of resorufin) of the treatment wells could be read using a UV-Vis spectrophotometer. From these data, percent cytotoxicity or percent cell proliferation can be calculated. Significant differences between treatments could then be determined and post-hoc comparisons of the means could be done to evaluate which treatments are not significantly different from the controls. In the absence of a spectrophotometer, I have developed a scoring system based on the resulting color changes to compare different treatments (Table 1).

Safety issues

*S. cerevisiae* is a BSL1 organism, and all work should thus be performed in BSL1 laboratories, with appropriate personal protective equipment. Students should be trained in BSL1 procedures prior to conducting this laboratory activity. During the creation and use of these protocols, all ASM biosafety guidelines were followed (https://www.asm.org/index.php/educators/laboratory-safety-guidelines).

CONCLUSION

This activity that I have developed is a simple, cheap alternative to conventional cell viability testing techniques commonly employed in research. It is therefore recommended for use in institutions with limited budgets to acquire materials for instruction purposes. I have tried this method with a group of students to test the cellular proliferative effects of the ethanolic extracts of some tropical weeds (example shown in Fig. 1), with positive results.

SUPPLEMENTAL MATERIALS

Appendix 1: Preparation of resazurin solution
Appendix 2: Preparation of yeast suspension
Appendix 3: Suggested treatments
Appendix 4: Protocol for cell viability assay

TABLE 1. System of scoring developed for the observation of color change to compare the cellular effects of the treatments.

| Color          | Score | Cellular Effect                  |
|----------------|-------|----------------------------------|
| Blue           | 0     | Cells are dead                   |
| Dark violet    | 1     | Cells are alive but some are dying|
| Violet         | 2     | Cells are alive but no proliferation|
| Light violet   | 3     | Cells have proliferated slowly   |
| Pink           | 4     | Cells have proliferated moderately|
| Light pink     | 5     | Cells have proliferated fast     |
| Very light pink| 6     | Cells have proliferated very fast|

FIGURE 1. Sample results of RRT in *S. cerevisiae* treated with 0.1 M CdCl2 (a), sterile distilled water (b), tropical weed ethanolic extract concentrations of 100 ppm (c), 250 ppm (d), and 500 ppm (e), and 1% glucose solution (f).
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