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Vybrant™ Cell Metabolic Assay Kit (V-23110)

Quick Facts

Storage upon receipt:
- –20°C
- Protect from light

Abs/Em of reaction product: 563/587 nm

Introduction

Nonfluorescent resazurin (R-12204), which can be reduced by viable cells to red-fluorescent resorufin, has been extensively used to detect the metabolic activity of many different cell types, from bacteria to higher eukaryotes.[1-3] Resazurin is nontoxic and stable in culture media, allowing researchers to continuously monitor proliferating cells[4] and to investigate cytotoxicity in both conventional[5] and high-throughput applications.[6]

The Vybrant™ Cell Metabolic Assay Kit (V-23110) includes lipophilic C_{12}-resazurin, which surpasses resazurin in cell permeability. The reduction product of this modified resazurin (C_{12}-resorufin) exhibits enhanced cellular retention (Figure 1), which results in brighter signals and better detection limits. C_{12}-resazurin may be used in any assay that employs resazurin (also called alamarBlue, a trademark of AccuMed International, Inc.). Because C_{12}-resorufin has the same absorption and emission maxima (563/587 nm, respectively) as unmodified resorufin, no instrumentation changes are required to use the Vybrant Cell Metabolic Assay Kit in place of a standard resazurin-based assay. For convenience, this kit also contains anhydrous DMSO for dissolving the C_{12}-resazurin, and resorufin for generating standard curves.

Materials

Kit Contents

- **C_{12}-Resazurin** (MW = 398, Component A), 5 vials, each containing 80 µg of lyophilized powder
- **Dimethylsulfoxide (DMSO), anhydrous** (Component B), 1 vial containing 200 µL of high-quality anhydrous DMSO
- **Resorufin, sodium salt** (MW = 235, Component C), 1 vial containing 47 µg of lyophilized powder

Storage and Handling

Upon receipt, components should be stored at -20°C until required for use; avoid repeated freezing and thawing. When stored properly, the kit components should be stable for at least six months.

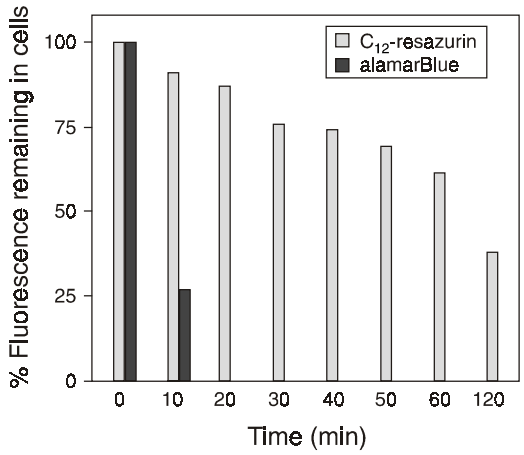

Figure 1. *Loss of signal from cells loaded with either C_{12}-resazurin or alamarBlue. Equal numbers of Jurkat cells were loaded for 15 minutes with 10 µM of C_{12}-resazurin, or for 30 minutes with 25 µM alamarBlue at 37°C. Cells were washed twice with cold PBS (pH 7.4) and incubated at 37°C. At the indicated times, samples were removed. The cells were collected by centrifugation, fixed with 4% paraformaldehyde/PBS and transferred to 96-well microplates for analysis. Not only does alamarBlue leak from cells at a much faster rate than C_{12}-resazurin, but it must be loaded at a higher concentration and for a longer time to achieve comparable initial fluorescence intensity.*

Experimental Protocol

Stock Solution Preparation

Allow all kit components to warm to room temperature before preparing the stock solutions. Prepare a 10 mM stock solution of C_{12}-resazurin by dissolving the contents of one vial of C_{12}-resazurin (Component A) in 20 µL of DMSO (Component B). It may be necessary to agitate the solution in an ultrasonic water bath to fully dissolve the C_{12}-resazurin. The C_{12}-resazurin stock solution should be stable for three months if stored at -20°C, protected from light.

The resorufin included in this kit (Component C) can be used to generate a standard curve to determine the moles of C_{12}-resorufin produced by the reduction of C_{12}-resazurin. If a standard curve will be generated, prepare a 2 mM stock solution of resorufin by adding 100 µL of dH_2O directly to the vial of resorufin (Component C). This stock solution should be stored frozen at -20°C, protected from light.

Cell Loading Guidelines

The appropriate probe concentration for optimal staining will vary by application. The initial conditions suggested here are intended as guides but may need to be modified depending on the

cell type and the permeability of the cells or tissues to the probe, among other factors. The 10 mM stock solution of C_{12}-resazurin (see Stock Solution Preparation) may be diluted in the desired culture medium or buffer. However, media containing thiols (e.g., dithiothreitol or cysteine) should be avoided to prevent further reduction of the fluorescent C_{12}-resorufin. For microplate-based assays, loading concentrations of 5–10 µM are recommended. For flow cytometry applications, the loading concentration should be reduced to 0.1–0.5 µM. For either type of application, incubate the cells for 15 minutes at 37°C.

Standard Curve for Microplate Assays

If a standard curve is desired, dilute the appropriate amount of 2 mM resorufin stock solution (see Stock Solution Preparation) into the same culture medium or buffer used for the experiment to yield resorufin solutions ranging from 0 to 20 µM resorufin. For each standard, pipet a volume equivalent to that used for the experimental wells into individual empty microplate wells at any time prior to measuring the fluorescence.

References

1. Appl Environ Microbiol 56, 3785 (1990); **2.** J Dairy Res 57, 239 (1990); **3.** J Neurosci Methods 70, 195 (1996); **4.** J Immunol Methods 210, 25 (1997); **5.** J Immunol Methods 213, 157 (1998); **6.** Antimicrob Agents Chemother 41, 1004 (1997).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
V-23110	Vybrant™ Cell Metabolic Assay Kit *with C_{12}-resazurin* *500-1000 assays*	1 kit

Contact Information

Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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