**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

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**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. Resazurin is nontoxic and stable in culture media, allowing researchers to continuously monitor proliferating cells and to investigate cytotoxicity in both conventional and high-throughput applications.

The Vybrant™ Cell Metabolic Assay Kit (V-23110) includes lipophilic C₁₂-resazurin, which surpasses resazurin in cell permeability. The reduction product of this modified resazurin (C₁₂-resorufin) exhibits enhanced cellular retention, resulting in brighter signals and better detection limits. C₁₂-resazurin may be used in any assay that employs resazurin (also called alamarBlue, a trademark of AccuMed International, Inc.). Because C₁₂-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 C₁₂-resazurin, and resorufin for generating standard curves.

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**Materials**

**Kit Contents**
- **C₁₂-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.

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**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 C₁₂-resazurin by dissolving the contents of one vial of C₁₂-resazurin (Component A) in 20 µL of DMSO (Component B). It may be necessary toagitate the solution in an ultrasonic water bath to fully dissolve the C₁₂-resazurin. The C₁₂-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 C₁₂-resorufin produced by the reduction of C₁₂-resazurin. If a standard curve will be generated, prepare a 2 mM stock solution of resorufin by adding 100 µL of dH₂O 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 C₁₂-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 C₁₂-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


### Product List

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

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<td>V-23110</td>
<td>Vybrant™ Cell Metabolic Assay Kit <em>with C₁₂-resazurin</em> <em>500-1000 assays</em></td>
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