Alamar Blue Cell Viability Assay Kit

Introduction

The Alamar Blue Cell Viability Assay Kit is a kit that detects cell proliferation by the redox indicator Resazurin. Resazurin is oxidized, which is blue in color and basically non-fluorescent. Once entering the cells, resazurin is reduced to resorufin. Resorufin is pink in color and highly red fluorescent. The number of living cells is proportional to the fluorescent or colorimetric signal of resorufin. Therefore, cell viability can be detected by fluorescence- or colorimetric-based instrumentation. But colorimetric detection is not as sensitive as fluorescence detection.

This kit has a wide range of applicability for the proliferation assay of animal cells, plants, bacteria, and fungi. Compared with MTT, WST-1, CCK-8 and other methods, this kit has higher sensitivity, and can detect as low as 50-100 cells per well when using fluorescence. Moreover, this kit is a ready-to-use reagent that is suitable for high-throughput screening. In addition, this reagent is non-toxic and does not affect cell health. For 96-well plates, 5 mL of this reagent is supplied for 500 assays; 25 mL is supplied for 2500 assays; and 100 mL is supplied for 10000 assays.

Components and Storage

Components	K2234-5 mL	K2234-25 mL	K2235-100 mL
Alamar Blue ready-to-use solution	5 mL	25 mL	100 mL
Store this reagent at 4°C away from light, stable for 6 months.			

Protocol

1. **Cell culture:** Seed cells in a 96-well plate with 100 µL cell suspension per well. For adherent cells, 100-10000 cells per well are recommended; for suspension cells, 2000-50000 cells per well are recommended.

***Note:** Black 96-well cell culture plates are recommended for fluorescence detection.

2. **Drug treatment:** Cells are treated with the tested drug according to the experimental design. To detect the effect of the drug on cell proliferation, different groups should be set up in the experiment. Set up wells with culture medium without cells to serve as the background control; set up wells with untreated cells to serve as the negative control; set up wells with treated cells to serve as the experimental group.

3. **Alamar Blue Incubation:** For 96-well plates, add 10 µL Alamar Blue ready-to-use solution per well. Incubate

at 37°C protected from light for 1-4 h. The incubation time can be adjusted according to the specific experiment.

***Note:** For samples with fewer cells, it is suggested to use longer incubation time.

4. **Detection:** After incubation, measure Fluorescence at Ex/Em = 570/595 nm.

***Note:** Fluorescence is the preferred method because it is more sensitive and needs fewer data calculations than colorimetric detection.

5. **Calculate cell viability:**

For fluorescence detection, the calculation method is as follows:

$$\text{Cell viability (\%)} = 100 \times (F_{\text{Experimental group}} - F_{\text{Background control}}) / (F_{\text{Negative control}} - F_{\text{Background control}})$$

***Note:** F is the fluorescence intensity (Ex/Em: 570/595).

Note

1. In order to obtain the ideal experimental results, it is recommended to do a pre-experiment to explore the optimal cell seeding number and optimal incubation time before the experiment.
2. If the cell seeding number is too much or the incubation time is too long, a secondary reduction reaction may occur, resulting in colorlessness or no fluorescence.
3. Although this reagent can also be detected by a colorimetric method, fluorescence detection is more sensitive and preferred.
4. When detecting animal cell proliferation, the experimental procedure needs to be sterile, as bacteria can also react with the Alamar Blue reagent.
5. For your safety and health, please wear a lab coat and disposable gloves.
6. This product is for scientific research use only and should not be used for clinical diagnosis or treatment.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com