Alarm-Blue 细胞增殖与活性检测试剂说明书

产品概述

Alamar-Blue 检测试剂为细胞增殖和细胞毒性检测提供了一种简便、快速、可靠、安全的方法，适用于高通量检测实验。该检测试剂的主要成分是一种氧化还原指示剂。其在氧化状态下呈现紫蓝色无荧光性，而在还原状态下，转变为呈粉红或红色荧光的还原产物，其吸收峰为 530-560nm，而发射峰为 590nm。

在细胞增殖过程中，细胞内 NADPH/NADP、 FADH/FAD、 FMNH/FMN 和 NADH/NAD 的比值升高，处于还原环境。摄入细胞内的染料被这些代谢中间体及细胞色素类还原后释放到细胞外并溶于培养基中，使培养基从无荧光的靛青蓝变成有荧光的粉红色。最后用普通分光光度计或荧光光度计进行检测，吸光度和荧光强度与活性细胞数成正比。

本产品无细胞毒性，不影响细胞代谢、细胞因子分泌、抗体合成等，检测后的细胞仍然可以进行后续实验。其适用于细菌、酵母类、昆虫类、鱼类、哺乳类等多种细胞，以及贴壁细胞与半贴壁细胞的检测，可以广泛用于细胞增殖、细胞毒性以及病原微生物的快速检测与鉴定。

包装规格

产品编号	091-5	091-25	091-100
Alamar-Blue 细胞增殖与活性检测试剂（无菌）	5mL	25mL	100mL
说明书	1 份		

使用方法

在待测样品中加入 10%细胞悬液体积的检测试剂，在细胞培养箱内孵育 2-6 小时，培养基的颜色由靛青蓝开始变成粉红色就可以进入下一步。

推荐使用荧光酶标仪进行检测，激发光波长在 530-560 nm 之间，发射光波长为 590 nm，记录相对荧光单位（RFU）。

绘制标准曲线或细胞生长曲线：纵座标（Y 轴）为相对荧光单位（RFU）；横坐标（X 轴）为细胞数或时间点或药物浓度。

储存方法

2-8℃避光保存，请在 20 个月内使用。

注意事项

合适密度的细胞可以增加检测灵敏度。对于 96 孔板，我们建议每孔接种 100 微升细胞，细胞浓度范围为：贴壁细胞在 100-10,000/孔，悬浮细胞在 2,000-50,000/孔，并以培养基为空白对照。对于 384 孔板，细胞浓度和接种量均减半。

整个过程均应为无菌操作，因为微生物污染物同样可以还原检测试剂而影响实验结果。

注意接种细胞浓度和加入检测试剂后孵育时间。细胞浓度过高或孵育时间过长，会导致继发性还原反应，产生无色和荧光消失。

孵育时，须避光。

本产品可以使用荧光或分光光度检测，但荧光的灵敏度高，实验误差小，推荐使用荧光检测

AlarmarBlue Cell Viability Reagent

Introduction

Cell health can be monitored by numerous methods. Plasma membrane integrity, DNA synthesis, DNA content, enzyme activity, presence of ATP, and cellular reducing conditions are known indicators of cell viability and cell death. AlarmarBlue cell viability reagent functions as a cell health indicator by using the reducing power of living cells to quantitatively measure the proliferation of various human and animal cell lines, bacteria, plant, and fungi allowing you to establish relative cytotoxicity of agents within various chemical classes. When cells are alive they maintain a reducing environment within the cytosol of the cell. Resazurin, the active ingredient of AlarmarBlue reagent, is a non-toxic, cell permeable compound that is blue in color and virtually non-fluorescent. Upon entering cells, resazurin is reduced to resorufin, a compound that is red in color and highly fluorescent. Viable cells continuously convert resazurin to resorufin, increasing the overall fluorescence and color of the media surrounding cells.

AlarmarBlue cell viability reagent is used to assess cell viability by simply adding the 10X, ready-to-use solution to mammalian or bacterial cells in culture media. There is no requirement to aspirate media from cells or place cells in minimal media. Consequently, AlarmarBlue reagent can easily be used in a single tube or microtiter plate format in a “no wash” fashion. Simply add AlarmarBlue reagent as 10% of the sample volume (i.e., add 10 µL AlarmarBlue reagent to 100 µL sample), followed by a 1–4 hours incubation at 37ºC. Longer incubation times may be used for greater sensitivity without compromising cell health. The resulting fluorescence is read on a plate reader or fluorescence spectrophotometer. Alternatively, the absorbance of AlarmarBlue reagent can be read on a spectrophotometer. Finally, results are analyzed by plotting fluorescence intensity (or absorbance) versus compound concentration.

Materials required but not provided

Mammalian or bacterial cells in appropriate medium
Appropriate 96- or 384-well plates
Optional: 3% SDS in phosphate buffered saline (PBS), pH 7.4

Preparing Cells

Mammalian Cells—Adherent: Plate mammalian cells in a cell culture flask or dish, and allow cells to adhere and grow for approximately 4–24 hours at 37°C and 5% CO2 before proceeding with the assay.
Mammalian Cells—Suspension: Plate mammalian cells in a cell culture flask or dish, and use cells immediately for the assay or allow cells to grow for up to 24 hours at 37°C and 5% CO2 before proceeding with the assay.
Bacterial Cells: For details, see references 2 and 3. Notes AlarmarBlue reagent is stable to multiple freeze/thaw cycles and its activity is not affected if the reagent is frozen.

General Guidelines

Cell types assayed with AlarmarBlue reagent include mammalian, bacterial (including biofilms), plant, and fish cells. More specifically AlarmarBlue reagent has been tested on hepatocytes, such as HepG2 cells, as well as cells of primary origin.

Be sure to include appropriate assay controls. To minimize experimental errors, we recommend making measurements from a minimum of 4–8 replicates of experimental and no-cell control samples.

You may need to determine the plating density and incubation time for the AlarmarBlue assay for each cell type and use conditions such that the assay is in the linear range.

If you plan to use longer incubation time (overnight), be sure to maintain sterile conditions during reagent addition and incubation to avoid microbial contaminants. Contaminated cultures will yield erroneous results as microbial contaminants also reduce AlarmarBlue reagent.

Fetal bovine serum (FBS) and bovine serum albumin (BSA) cause some quenching of fluorescence. We recommend using the same serum concentration in controls to account for this quenching. Other media components, such as phenol red do not interfere with the assay.

Protocol

Optional: Treat cells with the test compound 24–72 hours prior to performing the AlarmarBlue cytotoxicity assay.
Add 1/10th volume of AlarmarBlue reagent directly to cells in culture medium.

Incubate for 1 to 4 hours at 37°C in a cell culture incubator, **protected from direct light**.
Note: Sensitivity of detection increases with longer incubation times. For samples with fewer cells, use longer incubation times of up to 24 hours.

Record results using fluorescence or absorbance as follows:
Fluorescence: Read fluorescence using a fluorescence excitation wavelength of 540–570 nm (peak excitation is 570 nm). Read fluorescence emission at 580–610 nm (peak emission is 585 nm).
Absorbance: Monitor the absorbance of AlarmarBlue at 570 nm, using 600 nm as a reference wavelength (normalized to the 600 nm value).
Note: Fluorescence mode measurements are more sensitive. When fluorescence instrumentation is unavailable, monitor the absorbance of AlarmarBlue reagent. Assay plates or tubes can be wrapped in foil, stored at 4°C, and read within 1–3 days without affecting the fluorescence or absorbance values.

Optional: Add 50 µL 3% SDS directly to 100 µL of cells in AlarmarBlue reagent to stop the reaction.

References

1. Invest Ophthalmol Vis Sci 38, 1929 (1997); 2. Infect Immun 65, 3193 (1997); 3. J Antimicrob Chemother 57, 1100 (2006) ; 4. Phytochem Anal 12, 340 (2001) ; 5. Anal Biochem 344, 76 (2005)

FAQ's

General Questions

Q: How does AlarmarBlue work?
A: Healthy living cells maintain a reducing state within their cytosol. This “reducing potential” of cells converts AlarmarBlue reagent into a detectable fluorescent (or absorbent) product.

Q: Is AlarmarBlue reagent toxic?
A: No. AlarmarBlue reagent is a safe, non-toxic reagent to both the sample and user.

Q: Does AlarmarBlue reagent need reconstitution?
A: No, AlarmarBlue reagent is supplied as a 10X, ready-to-use solution.

Q: Can I use AlarmarBlue reagent with suspension cells too?
A: Yes. AlarmarBlue reagent works on adherent and suspension mammalian cells.

Q: Can I use AlarmarBlue reagent with non-mammalian cells, such as bacteria?
A: Yes, AlarmarBlue reagent has been shown to work with bacterial 2 and plant cells. 4

Q: AlarmarBlue reagent is not the most expensive cytotoxicity indicator on the market, does that mean it doesn't work as well as other reagents?
A: Actually, AlarmarBlue reagent is comparable to other often more expensive cytotoxicity indicators.

Q: Since AlarmarBlue is an absorbance or fluorescence readout, is it as sensitive as a luminescence product?
A: AlarmarBlue reagent is sensitive enough to detect less than 50 mammalian cells in a single well of a 96-well plate.

Storage Questions

Q: What if I left the AlarmarBlue stock reagent at room temperature, overnight?
A: The reagent is stable for up to 12 months when stored at room temperature (~22°C).

Q: I accidentally froze the AlarmarBlue stock reagent, can I still use it?
A: Yes. AlarmarBlue reagent is stable to multiple freeze/thaw cycles. Be sure to heat the reagent in a 37°C water bath and mix the reagent to ensure a homogenous solution before use.

Q: Do I need to protect AlarmarBlue reagent from light?
A: Yes, AlarmarBlue reagent is very slowly converted into a fluorescent product over time, when exposed to light, thus leading to high background values. Store the reagent, protected from light.

Methods Questions

Q: What is the optimal incubation time and temperature of cells with AlarmarBlue reagent?
A: Incubate the cells with AlarmarBlue reagent for 1–4 hours at 37°C. For more sensitive detection with low cell numbers, increase the incubation time for up to 24 hours.

Q: Can you incubate cells with AlarmarBlue reagent overnight?
A: Yes. However, signals from higher cell density samples may have “saturated,” which means the linearity of reagent may have reached a plateau. If this occurs, decrease the incubation time.

Q: What if I don’t have an instrument suitable for reading fluorescence?
A: The absorbance of AlarmarBlue reagent also changes depending on cell viability and proliferation. Therefore, simply monitor the absorbance of the reagent at 570 nm, while using 600 nm as a reference wavelength.

Q: Is AlarmarBlue assay strictly an endpoint assay?
A: No. While AlarmarBlue can be used as a terminal readout of a population of cells, the reagent can also be used to continuously monitor cell viability and proliferation in real time. Since AlarmarBlue reagent is non-toxic, you can incubate cells with reagent and monitor fluorescence (or absorbance) over time on the same sample.

Troubleshooting Questions
Q: What is the problem for observing high background fluorescence values?
A: The reagent may be breaking down due to exposure to light. Be sure to store AlarmarBlue reagent in the dark and do not expose the reagent to direct light for long periods of time.
Q: Why are the fluorescence values so low in intensity?
A: Try increasing the incubation time of cells with AlarmarBlue reagent, changing the instrument’s “gain” setting, and checking the instrument filter/wavelength settings. Make sure to have positive controls (living cells) in the experimental design for troubleshooting.
Q: Why are the fluorescence values so high that they are beyond the linear range of the instrument?
A: Try decreasing the incubation time or reducing the number of cells used in the experiment.