Alamar Blue 细胞增殖与活性检测盒
Alamar Blue 细菌活性检测试剂盒

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。能根据代谢活性产生吸亮度变化和荧光信号。这是一种安全无毒的染料，可用于细胞活性和细胞增殖的定量分析以及细胞因子生物测定和体外细胞毒性研究，能有效测定动物、真菌和细菌细胞的天然代谢活性。Alamar Blue 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，反映所研究的细胞或微生物对氧分子的消耗，因而常被用作氧化还原指示剂，以显示被观察细胞和细菌的代谢活动。这种测定是基于具有代谢活性的细胞将试剂转换成荧光和比色指示剂的能力。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。受损和无活性细胞具有较低的天然代谢活性，因此对应的信号较低。可以用普通分光光度计或荧光光度计检测，其激发光波长在 530-560nm 之间，发射光波长为 590nm。吸光度和荧光强度与活性细胞数成正比。

本品可广泛地用于细胞增殖代谢，药物的细胞毒作用的体外测定以及病原微生物的的快速检测与鉴定。与台盼兰、TTC、MTT、MTS 等分析法相比，Alamar Blue 具有更多的优势。Alamar Blue 采用单一试剂，可以连续、快速地检测细胞的增生状态。由于 Alamar Blue 对细胞无毒、无害，不影响细胞的抗体合成与分泌等活性，因此可以对同一批细胞的增生状态进行连续观察和进一步的实验观察，因此有操作简便和几乎不干扰细胞正常代谢，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，以及贴壁细胞与非贴壁细胞的检测，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。

产品特点

1. 使用方便：仅使用单一试剂
2. 对微生物细胞进行灵敏、无毒且安全的细胞活力和增殖测定；
3. 能长期使用，持续进行测定而不损伤细菌或产生有毒废物；
4. 灵活、简便- 能与多种仪器平台(酶标仪或分光光度计)、96 和 384 孔板以及荧光或分光光度法测定兼容

包装规格

产品编号	JXR002-5	JXR002-25	JXR002-100
Alamar Blue 细胞增殖与活性检测试剂（无菌）	5mL	25mL	100mL
说明书	1 份		

储存方法 2-8℃避光保存，请在 24 个月内使用。

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。酶标仪带有 450nm 滤光片。

推荐使用荧光酶标仪进行检测，激发光波长在 530-560nm 之间，发射光波长为 590nm，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。

操作说明

一、制作标准曲线（测定最佳孵育时间和细胞数量）

1 每孔加入 100 微升细胞悬液（对数生长期细胞），对细胞计数。按比例依次用培养基等比稀释成一个细胞浓度梯度，一般要做 3-5 个细胞浓度梯度，每个浓度建议 3-6 个复孔。注：设置阴性对照：细胞培养基中不加入细胞；阳性对照： 100 微升 100%还原型Alamar Blue（不含细胞）。

2 每孔加入10 微升Alamar Blue。阳性对照孔中加入10微升无菌的超纯水。

3 放入细胞培养箱内培养（37℃,5% CO_2）一定时间。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。

4 推荐用荧光分光光度计检测，激发光波长在 530-560 nm 之间，发射光波长为 590 nm。

5 普通分光光度计在 570 nm 测定吸光度，参考波长 600nm。也可用 570/630 nm 和 540/600 nm 替代。

6 绘制标准曲线。以细胞数量或孵育时间为横坐标（X 轴），Alamar Blue 还原率为纵坐标（Y 轴）。根据此标准曲线可以测定出适合的细胞数量或孵育时间（使用此标准曲线的前提是实验的条件要一致）。

二、细胞毒性和细胞增殖检测

1 每孔加入 100 微升细胞悬液（对数生长期细胞），对细胞计数。建议细胞数量为 10^4/mL，具体每孔需要的细胞数量，需根据细胞类型决定。

2 细胞中加入待检测药物，为检测药物对细胞增殖的作用，请设置合适的对照组，包括刺激细胞和非刺激细胞。

3 混匀，放入细胞培养箱内孵育（37℃,5% CO_2）一段时间。

4 每孔加入10微升Alamar Blue。阳性对照孔中加入10微升无菌的超纯水。

5 放入细胞培养箱内孵育（37℃,5% CO_2）4-8 h，最佳孵育时间取决于细胞类型。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。

6 推荐用荧光分光光度计检测，激发光波长在 530-560 nm 之间，发射光波长为 590 nm。

7 普通分光光度计在 570nm 测定吸光度，参考波长 600 nm。也可用 570/630 nm 和 540/600 nm 替代。

活力计算

1. 普通分光光度计检测

Alamar Blue 还原率（%）=[（E600×A570）-（E570×A600）]/[(E570′ ×C600)-E600′ ×C570] ×100

E570=氧化型 Alamar Blue 在 570 nm 波长的消光系数=80586；

E600=氧化型 Alamar Blue 在 600 nm 波长的消光系数=117216

A570=检测孔在 570 nm 波长的吸亮度；A600=检测孔在 600 nm 波长的吸亮度；

E570′ =还原型 Alamar Blue 在 570 nm 波长的消光系数=155677；

E600′ =还原型 Alamar Blue 在 600 nm 波长的消光系数=14652；

C570=阴性对照孔在 570 nm 波长的吸亮度（培养基，Alamar Blue，无细胞）

C600=阴性对照孔在 600 nm 波长的吸亮度（培养基，Alamar Blue，无细胞）；

如果使用 570/630 nm 和 540/600 nm 作为替代，则：

E540=氧化型 Alamar Blue 在 540 nm 波长的消光系数=47619

E630=氧化型 Alamar Blue 在 630 nm 波长的消光系数=34798

E540′ =还原型 Alamar Blue 在 540 nm 波长的消光系数=104395

E630′ =还原型 Alamar Blue 在 630 nm 波长的消光系数=5494；

2. 荧光分光光度计检测

Alamar Blue 还原率（%）=(实验组 F590-阴性对照组 F590)/(100%还原型阳性对照 F590-阴性对照组 F590)×100 F590 波长荧光值

注意事项

1 需客户自行准备 100%还原型 Alamar Blue 试剂。为得到还原型 Alamar Blue，需配制 Alamar Blue 与细胞培养基的混合溶液，Alamar Blue 与细胞培养基的比值为 1:10，高压灭菌 15 min。（不能对浓缩的 Alamar Blue 高压灭菌，也不能用 PBS 配制溶液，因为 100%还原型 Alamar Blue 在PBS 中不稳定。）

2 孵育时，须避光！为得到最好的结果，建议实验前先摸索孵育时间和接种细胞数量。

3 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。

4 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Alamar Blue检测试剂而影响实验结果。培养基中应添加抗生素如青霉素G（1U/ml）、两性霉素B（amphotericin B，0.0025ug/ml）

5 Alamar BluepH敏感的，推荐使用磷酸缓冲液。10%胎牛血清对比色法检测无影响，但是会淬灭部分荧光；因此，用荧光法检测时对照溶液中需添加10%BSA。酚红对检测也有一定影响，结果会上调0.03%。请使用非还原性的培养基，如RPMI1640、Hank’s－Eagle或 Dulbecco’s－Eagle。

6 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。

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

三、 细菌活性检测

1) 正式实验前，建议先做预实验摸索接种细菌的数量和加入Alamar Blue试剂后的培养时间。

2) 细菌的活性实验，一般可在96孔细胞培养板中进行，下面以96孔板检测为例，如果使用其他培养方法，AlamarBlue试剂加入培养液用量的10%即可。合适密度的细菌可以增加检测灵敏度

3) 每孔加入100微升细胞悬液。细胞的数量取决于实验目的和培养时间。具体每孔所用的细胞的数目，需根据细胞的大小，细胞增殖速度的快慢等因素确定。注意接种细菌浓度和加入检测试剂后孵育时间。细菌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。

4) 接种的细菌按照实验需要，送入培养箱进行培养。

5) 培养结束后，取出AlamarBlue试剂，置室温融化混匀，于洁净工作台内按10微升/孔加入微孔板中，在培养箱内继续孵育12－24小时，培养液颜色由蓝变为粉红（如采用荧光分光光度法，只需孵育2－8小时）

6) 在570nm测定吸光度，参考波长600nm。如无此滤光片， 可用565 nm 和 610 nm的滤光片替代。

7) 也可用荧光分光光度法检测，激发光波长560nm（在530-570 nm之间均可），发射光波长为590 nm。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。

8) 活性计算参考以上细胞的方法。

Alamar Blue 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. Alamar Blue 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 Alamar Blue 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.

Alamar Blue 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, Alamar Blue reagent can easily be used in a single tube or microtiter plate format in a “no wash” fashion. Simply add Alamar Blue reagent as 10% of the sample volume (i.e., add 10 µL Alamar Blue 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 Alamar Blue 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 Alamar Blue 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 Alamar Blue reagent include mammalian, bacterial (including biofilms), plant, and fish cells. More specifically Alamar Blue 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 Alamar Blue 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 Alamar Blue 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 Alamar Blue cytotoxicity assay.
Add 1/10th volume of Alamar Blue 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 Alamar Blue 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 Alamar Blue 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 Alamar Blue 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 Alamar Blue work?
A: Healthy living cells maintain a reducing state within their cytosol. This “reducing potential” of cells converts Alamar Blue reagent into a detectable fluorescent (or absorbent) product.

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

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

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

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

Q: Alamar Blue 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, Alamar Blue reagent is comparable to other often more expensive cytotoxicity indicators.

Q: Since Alamar Blue is an absorbance or fluorescence readout, is it as sensitive as a luminescence product?
A: Alamar Blue 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 Alamar Blue stock reagent at room temperature, overnight?
A: The reagent is stable for up to 12 months when stored at room temperature (~22ºC).

Q: I accidentally froze the Alamar Blue stock reagent, can I still use it?
A: Yes. Alamar Blue 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 Alamar Blue reagent from light?
A: Yes, Alamar Blue 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 Alamar Blue reagent?
A: Incubate the cells with Alamar Blue 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 Alamar Blue 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 Alamar Blue 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 Alamar Blue assay strictly an endpoint assay?
A: No. While Alamar Blue 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 Alamar Blue 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 Alamar Blue 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 Alamar Blue 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.