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# AlamarBlue Assay for Testing Cell Proliferation on PureCol® Encapsulated Cells

## Assay Description

The alamarBlue assay is a widely used and versatile method for assessing cell viability and proliferation. It is based on the reduction of the non-fluorescent compound resazurin to the fluorescent compound resorufin by metabolically active cells. This reduction process indicates the presence of living cells and can be quantitatively measured using fluorescence or absorbance techniques [1-3].

The alamarBlue assay is non-toxic, simple and highly sensitive, capable of detecting even small numbers of viable cells. This assay has been widely employed to characterize cell growth in various 3D cell culture platforms. Here we are using the alamarBlue assay to assess the proliferation of cells cultured in our PureCol® collagen hydrogels.

## Materials Needed

- PureCol® (Cat #5005)
- Cells/cell lines of interest
- Cell culture media
- alamarBlue™ Cell Viability Reagent (ThermoFisher, Cat #DAL1025)
- Cell culture plates
- Black 96-well plate
- 15 ml or 50 ml centrifuge tubes
- Tweezers or forceps
- Serological pipettes
- Micropipette and tips
- D-PBS
- Microplate reader

**REMINDER**
**All operations should be kept away from direct light exposure. Ensure lights are off in the biosafety cabinet while working with alamarBlue solution.**

## Procedure

### 1. Preparation of PureCol® solution and cell encapsulation

1.1. Slowly add 1 part of chilled 10X culture media to 8 parts of chilled collagen solution while gently swirling. Place the collagen mixture in the ice bucket to keep it cool.

1.2. Carefully adjust the pH of the collagen mixture to 7.0–7.5 using sterile 0.1 M NaOH. Monitor the pH using a pH meter, phenol red, or pH paper.

1.3. Adjust final volume to a total of 10 parts with sterile water.

1.4. Maintain the temperature of the mixture at 2 to 10 °C to prevent gelation.

1.5. Prepare and collect a cell pellet with the desired seeding number/density following instructions from the vendor of the cells. The cell pellet should stay at the bottom in the container (i.e. centrifuge tubes) with cell culture medium on top.

1.6. Gently remove the cell culture medium by aspiration and add chilled collagen solution to the cell pellet.

1.7. Gently mix the collagen solution the with the cells by pipetting the collagen solution using the serological pipette and pipettor. The cells should be well suspended in the collagen before plating.

1.8. Add cell-laden collagen solution to cell culture plates. Recommended seeding volume per well is listed below:

 

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Table 1. Cell seeding volume per well

| Plate size | Volume of Cell-Laden Collagen |
|---|---|
| 6 well | 1.5 mL |
| 12 well | 1 mL |
| 24 well | 0.5 mL |
| 48 well | 0.3 mL |
| 96 well | 0.1 to 0.2 mL |

1.9. Close the lid after dispensing the collagen solution and place the plates in an incubator (37 °C, 5% $CO_2$) to allow collagen gelation for 90 to 120 min.

1.10. Add cell culture medium to the plate and culture cells for desired culturing time.

**2. Alamarblue assay**

2.1. Warm up D-PBS and cell culture media in a 37 °C water bath.

2.2. Prepare 10% (v/v) alamarBlue working solution by adding alamarBlue stock solution into the corresponding cell culture medium. The total volume of the working solution is calculated based on the number and size of wells needed for the assay. For reference:

Table 2. alamarBlue reagent volume per well

| Plate size | Volume |
|---|---|
| 6 well | 2 ml |
| 12 well | 1.5 ml |
| 24 well | 1 ml |
| 48 well | 0.5 ml |
| 96 well | 0.3 ml |

Total working solution volume =∑ (Number of test wells + 1 blank well) × (amount of solution needed per well for each plate format)

** Prepare enough working solution plus extra in case of loss and spill during experiment operation.*

2.3. Aspirate the medium and add D-PBS into the well to rinse the collagen gels.

2.4. Remove D-PBS and add alamarBlue working solution to the test wells and the blank well. Solution volume per well refers to Table 2.

2.5. Incubate samples for 1.5~2.5 hr in the incubator.

** Exact incubation time is determined by the standard curve generated by cells/cell lines with a series of known cell number (i.e. 0, 10000, 20000, 50000, 75000, 100000, 200000, 500000, etc.) seeded in 2D wells. Monitor the color change during the incubation of the 2D cells. The optimal incubation time is determined when the highest cell number well changes color.*

2.6. After incubation, transfer 300 µl alamarBlue solution in duplicate from each well to the black 96-well plate.

2.7. Load the plate in the microplate reader and measure the fluorescence intensity at excitation wavelength of 560 nm and emission wavelength of 590 nm.

2.8. Convert the intensity into cell number using the standard curve generated by 2D cells.

**Reference:**

*[1] Page M, et al. "Evaluation of the Alamar Blue assay for measuring cell proliferation in high-throughput screening." Biotechnology Progress. 2007 Sep-Oct;23(5):1347-53.*
*[2] Rampersad SN. "Multiple applications of Alamar Blue as an indicator of metabolic function and cellular health in cell viability bioassays." Sensors. 2012;12(9):12347-60.*
*[3] O'Brien J, et al. "Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity." European Journal of Biochemistry. 2000 Apr;267(17):5421-6.*

 