Alarmablue Assay for Detecting Cell Viability

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[Abstract] AlamarBlue™ detects cell viability by utilizing a nonfluorescent dye resazurin, which is converted to a fluorescent dye resorufin in response to chemical reduction of growth medium resulting from cell growth. The fluorescent or colorimetric signal generated from the assay is proportional to the number of living cells in the sample (detailed information can be found from here).

Materials and Reagents

1. Resazurin cell viability assay kit (Biotium, catalog number: 30025)
2. Cells: in this protocol, three human prostate cancer cell lines are tested.
   a. DU 145 (ATCC, catalog number: HTB-81™)
   b. PC3 (ATCC, catalog number: CRL-1435™)
   c. LNCap (ATCC, catalog number: CRL-1740™)

Equipment

1. Spectra PLUS microplate reader
2. 96-well tissue culture plates

Procedure

A. Standard curve:

1. Plate cells in 100 µl medium into 96-well tissue culture plates by conducting cell number titration in the range of 40 to 10,000 for adherent cells and 2,000 to 500,000 for suspension cells. For background control, use 100 µl medium without cells.
2. Add 10 µl resazurin solution into medium and incubate cells at 37 °C overnight (between 1 to 24 h).
3. Measure absorbance at 570 nm and 600 nm using a micro-titer plate reader.
4. Obtain OD_{570}-OD_{600} for each sample and plot a standard curve to identify the optimal cell concentration for your assay.

B. Cell viability assay:

1. Plate cells into 96-well tissue culture plates using optimal cell concentration. For human prostate cancer cells DU145, PC3 and LNcap, 2,000 and 5,000 cells/well are used.
2. Carry out your experiment by adding agents of your interest into appropriate well and incubate with cells for a certain period of time.
3. Add 10 µl resazurin solution into medium and incubate cells at 37 °C overnight (between 1 to 24 h).
4. Measure absorbance at 570 nm and 600 nm using a micro-titer plate reader.
5. Obtain OD_{570}-OD_{600} for each sample and divide the OD value of test samples by that of control samples. Set control samples as 100% viable and calculate the cell viability.
   Cell viability = (OD of test samples)/ (OD of control samples) x 100%

References

1. Nociari, M. M., Shalev, A., Benias, P. and Russo, C. (1998). A novel one-step, highly sensitive fluorometric assay to evaluate cell-mediated cytotoxicity. *J Immunol Methods* 213(2): 157-167.

