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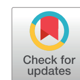
Protocol Article

Optimized alamarBlue assay protocol for drug dose-response determination of 3D tumor spheroids



Christoph Eilenberger[a,b], Sebastian Rudi Adam Kratz[a], Mario Rothbauer[a,*], Eva-Kathrin Ehmoser[b], Peter Ertl[a], Seta Küpcü[b]

[a] *Institute of Applied Synthetic Chemistry and Institute of Chemical Technologies and Analytics, Faculty of Technical Chemistry, Vienna University of Technology, Vienna Getreidemarkt 9/163, 1060 Vienna, Austria*
[b] *Institute of Synthetic Bioarchitectures, Department of Nanobiotechnology, University of Natural Resources and Life Sciences, Vienna, Muthgasse 11, 1190 Vienna, Austria*

A B S T R A C T

The assessment of drug-dose responses is vital for the prediction of unwanted toxicological effects in modern medicine. Three-dimensional (3D) cell cultures techniques can provide *in vivo*-like spheroids and microtissues that resemble natural tumor function. However, formation of necrotic core and diffusion limitation of chemical compounds within these models can reduce the reproducibility and precision of standard bioassay protocols used to test two-dimensional (2D) cell cultures. Nonetheless, the accurate prediction of detrimental effects of test compounds based on functional bioassays is essential for the development of new efficient therapeutic strategies. For instance, alamarBlue® is a widely-used commercially available redox indicator dye that can evaluate metabolic activity and cellular health status in a single-step procedure however, suitability and optimization of this bioassay must be determined for each individual application scenario. Here, we optimized the standard alamarBlue® proliferation/viability protocol for tumor spheroid cultures to enhance assay precision during toxicological drug screening.

We optimized the original protocol of alamarBlue® assay that usually suggests an incubation time of 2–4 hours. The key modifications of the protocol for spheroid cultures are as follows:

- Aspiration of cell culture medium before drug exposure.
- Replacement of drug-supplemented medium with 10% (v/v) alamarBlue® reagent mixed with culture medium.
- Increase of incubation period to 24 h at 37 °C protected from light.

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* Corresponding author.
E-mail address: mario.rothbauer@tuwien.ac.at (M. Rothbauer).

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Specifications Table

Subject area	• *Pharmacology, Toxicology and Pharmaceutical Science*
More specific subject area	*Cell biology, Tissue engineering*
Method name	*AlamarBlue proliferation assay*
Name and reference of original method	[1–3]
Resource availability	https://www.thermofisher.com/at/en/home/references/protocols/cell-and-tissue-analysis/cell-profilteration-assay-protocols/cell-viability-with-alamarblue.html

Method details

Preparation and generation of spheroid cultures

Materials

- Cell culture facility equipped with a CO_2 incubator, laminar flow hood, bright-field microscope, a centrifuge and a cell counter.
- Plastic consumables: cell culture dishes and flasks, serological pipettes, syringes and centrifuge tubes.
- Hepatocellular carcinoma cells (HepG2).
- Cell culture medium: Minimal essential medium supplemented with 10% v/v of fetal bovine serum, 1%vol. of 20 mM L-glutamine and 1%vol. of 100 mM penicillin and streptomycin.
- Dulbecco's Phosphate Buffered Saline (PBS) 1X (pH 7.1–7.4).
- Trypsin-EDTA solution (0.25%).
- Trypan blue stain 0.4%.

Procedure

Cells taken for experiments should be at log-phase of growth, approx. 60–80 % confluent. Amounts of media given for 75 cm^2 cell culture flasks. All media applied to cells should be pre-warmed to 37 °C. (*Note*: Spheroid cultivation time, as well as morphology, can vary for different cell types. Therefore, initial cell seeding density should be pre-screened to identify the optimal cell density.)

1 Remove medium from cell culture. Wash the cells with PBS.
2 Detach cells using 5 mL trypsin solution. Incubate for at least 5 min at 37 °C until cells detach from the surface.
3 Add 5 mL of cell culture medium.
4 For trypsin removal transfer the suspension into 15 mL Falcon tube and centrifuge for 5 min at 294×*g*.
5 Gently remove supernatant and add 5 mL fresh medium.
6 Push the cell solution through a needle by using a syringe to dissociate larger aggregates into individual cells.
7 Mix 10 μL cell solution and 10 μL Trypan Blue in an Eppendorf tube and transfer 10 μL of the mix to a cell counter slide.

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