# Measuring cell viability: metabolic activity assays vs. confluence measurements

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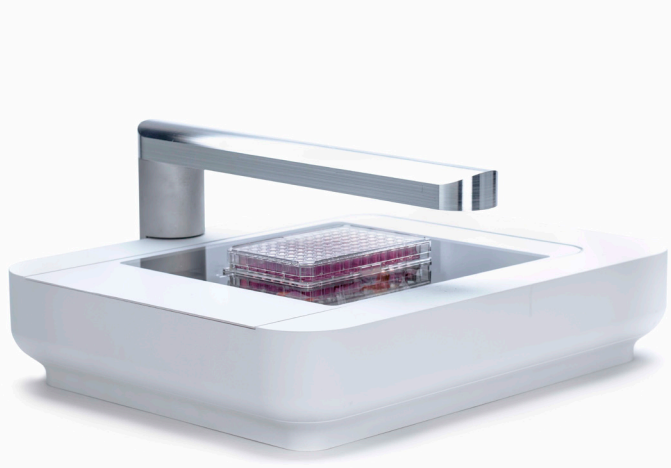
## Introduction

Cell viability is an important measure for many investigators working in the fields of cancer research, drug development and tissue engineering. Usually, metabolic activity of cells is taken as a cell viability measure. However, the major downside of metabolic activity assays (e.g. MTT and Alamar Blue assay) is that they are endpoint measurements. Thus, in case it is of interest to monitor viability over time, the number of samples needs to be increased to repeat the assay several times. Using non-invasive automated confluence measurements, the same samples can be followed over time, which results in more accurate data with less samples and resources. Using the CytoSMART® Omni, bright-field images of a complete well-plate can be made.

## Example

A PhD-candidate wants to investigate the effect of a drug (e.g Thapsigargin) on cell viability over time using a dose-response experiment containing 96 samples per timepoint (in a 96 well-plate). Preferably, he/she wants to determine the viability every 3 h for 24 h leading to 8 timepoints in total. Before starting the experiment, the cells need to be expanded until the right amount of cells is obtained to perform the experiment.
Below, we compare the costs and time consumption of the Alamar Blue assay with bright-field confluence measurements (with the CytoSMART® Omni). The calculations are based on the culture of C6 cells, the calculations could differ when using different cells.



## Expanding cells

Before starting the cell viability experiment, the cells need to be expanded. Since 8 96-well plates are necessary for the Alamar Blue assay, 8 times more cells are needed for this experiment compared to confluency measurements. Thus, when thawing the same amount of cells for both methods, a longer expansion time is needed for the Alamar Blue experiment compared to the confluency measurements. Furthermore, more consumables and media are needed.

### Costs

| | Alamar blue | Confluence measurements |
|---|---|---|
| Consumables (pipets, flasks, etc.) | € 9.90 | € 5.44 |
| Media (culture medium, PBS, trypsin) | €17.40 | € 5.30 |
| Total | €27.30 | €10.74 |

*Culture medium consists of DMEM supplemented with 10% FBS and 1% penicillin/streptomycin

### Time

| | Alamar blue | Confluence measurements |
|---|---|---|
| Thawing cells | ± 30 min | ± 30 min |
| Passaging cells | ± 30 min | - |
| Total | 1 h | 30 min |

N.B. The time reported in this table is the time actually spent in the lab. If the total expansion time is taken into account this changes to approximately 8 days (Alamar Blue) and 4 days (Confluence measurements).

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## Experiment optimization

Before starting the Alamar Blue measurements, the incubation time needs to be optimized as this depends on the cell line. To optimize this, the Alamar Blue intensity of the same well-plate needs to be measured at different incubation times (1-4 h).

### Costs

| | Alamar blue | Confluence measurements |
|---|---|---|
| Expansion (consumables and media) | €11.46 | - |
| 96-well plate (Corning) | € 2.50 | - |
| 9.6 ml culture medium | € 2.38 | - |
| 960 µl Alamar Blue reagent (Invitrogen) | € 4.92 | - |
| Total | €21.26 | - |

### Time

| | Alamar blue | Confluence measurements |
|---|---|---|
| Expansion (incl. thawing cells) | ± 30 min | - |
| Seeding samples | ± 45 min | - |
| Add reagent to samples | ± 15 min | - |
| Analysis of viability | ± 20 min (measure fluorescence) | - |
| Analysis of results | ± 60 min | - |
| Total | 2 h 50 min | - |

N.B. The time reported in this table is the time actually spent in the lab. If the expansion time (± 4 days), culture time (± 1 day), and incubation time (± 4 h) are taken into account, the total time to determine the optimum incubation time becomes 6 days.

## Experiment

The cell viability experiment itself consists of seeding the cells in 96-well plates (100 µl medium per well), culturing the cells until the desired confluency (e.g. 1 day), and replacing the medium by medium supplemented with the drug (100 µl per well). In this example 2 µl of Thapsigargin (Invitrogen) was used per 96 wells. Thereafter, the viability was measured every 3 h for 24 h.

### Costs

| | Alamar blue | Confluence measurements |
|---|---|---|
| 96-well plates (Corning) | 8 x €2.50 = € 20.00 | 1 x €2.50 = € 2.50 |
| 2x 9.6 ml culture medium per plate | 8 x €4.76 = € 38.08 | 1 x €4.76 = € 4.76 |
| Drug (2 µl Thapsigargin per well plate) | 8 x €7.10 = € 56.80 | 1 x €7.10 = € 7.10 |
| 960 µl Alamar Blue reagent (Invitrogen) | 8 x €4.92 = € 39.36 | - |
| Total | €154.24 | €14.36 |

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## Time

| | Alamar blue | Confluence measurements |
|---|---|---|
| Expansion (incl. thawing cells) | 1 x ± 90 min | ± 45 min |
| Seeding samples | 8 x ± 30 min | ± 30 min |
| Add reagent to samples | 8 x ± 15 min | - |
| Analysis of viability | 8 x ± 20 min (measure fluorescence) | ± 15 min (setting up the device) |
| Analysis of results | 1 x ± 60 min | ± 60 min |
| Total | 9 h 10 min | 2 h 30 min |

N.B. The time reported in this table is the time actually spent in the lab. If the culture time after seeding (± 1 day), and duration of the experiment itself (± 24 h) are taken into account, the total time becomes 3 days in both cases. However, since it is practically challenging to measure cell viability every 3 h in case the process is not automated, one could split the Alamar Blue experiment into two experiments (0-12 h and 12-24 h) which extends the total time to 4 or 5 days.

## Total savings

The costs and time consumption (actual time spend working in the lab) of the entire process, from thawing the cells to measuring their viability, are displayed in the tables below. Figure 1 also shows the amount of consumables, media and drugs necessary for each type of experiment. In total you could save approximately €178 in costs and 10.5 h in time per cell viability experiment.

## Costs

| | Alamar blue | Confluence measurements |
|---|---|---|
| Expansion of cells | € 28.02 | €11.46 |
| Optimization of experiment | € 21.26 | € 0.00 |
| Experiment | €154.24 | €14.36 |
| Total | €203.52 | €25.82 |

## Time

| | Alamar blue | Confluence measurements |
|---|---|---|
| Expansion of cells | 1 h 0 min | 0 h 30 min |
| Optimization of experiment | 2 h 50 min | 0 h 0 min |
| Experiment | 9 h 30 min | 2 h 30 min |
| Total | 13 h 20 min | 3 h 0 min |

In case this experiment is performed regularly in a research facility, e.g. 3 times per week for 40 weeks a year, the research facility can save up to approximately €22,000 a year in consumables, reagents, and drugs. Furthermore, the employees working in the research facility can save up to approximately 160 h a year, which equals 20 work days.

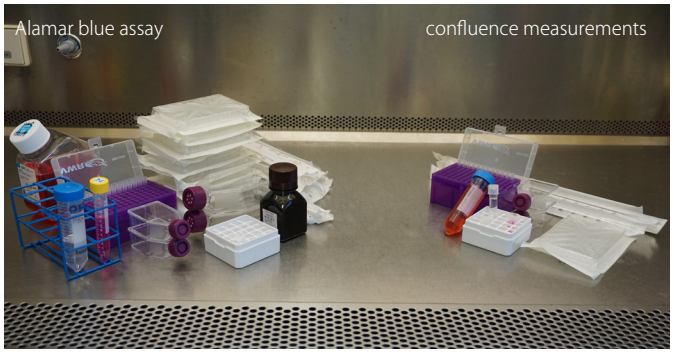


Figure 1. Difference in the amount of consumables, media and drugs necessary for the Alamar blue assay (left) and confluence measurements (right).