Resazurin viability assay for human primary T cells in 96-well format

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Abstract

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Guidelines

- Always make sure you have at least 4 replicates
- Always make sure to have blank media control (ideally on the same plate but having another plate with only media in it is OK, too)
- Plate readers are relatively less accurate for the most outer wells so do not use them specifically for a condition alone (it might bias your results). Having a replicate for each of your conditions in either of the most outer wells is OK.
- It is much easier to work in batches via multichannel pipettors and make use of the reagent cuvette
- The dye is sensitive to light/temperature. No need to go crazy about this but make sure you don’t leave the plate out for more than 10 minutes before measuring
- Do not go over 200 ul in total when preparing your cultures
- Plan carefully and remember to add the dye a day (24 hours) before your endpoint assay
- The assay is not that accurate > 300,000 cells per well or < 1,000 cells per well so make sure you seed the cells in such a way that you will hit the reliable cell counts on the day of your measurement

Before start

Pre-made Alamar Blue (Resazurin) solutions from companies (e.g. alamarBlue™ Cell Viability Reagent) are relatively expensive for long-term and common use. Therefore, ordering it in powder format is the cheapest and the most feasible way to go for extended use cases. 1 g powder (e.g. Resazurin sodium salt #R7017) should last for a year or two even if it is frequently used.

The final working concentration for Resazurin is 44 uM and our goal is to prepare a 10X solution for use in cell culture (e.g. for 200 ul of media/cells, we will add 20 ul of the dye). Directly preparing this is not feasible (due to the amount of PBS required) so we will first prepare a 1000X stock solution which we will further dilute as needed.

For 1000X Stock (44 mM):

- Weigh 5 gram of Resazurin powder
- Resolve the powder in 500 ml PBS (this will require vigorous shaking/mixing for a few times so make sure you don’t have any residues in the solution)

- Sterile filter the solution and prepare 10 aliquots (each 50 ml)
- Store the 1000X stocks at -20°C (either wrap each one with aluminum foil or use a cardboard box to limit light exposure for long term)

For 10X Stock (440 uM):

- Add 500 ul of 1000X stock onto 50 ml of PBS in a falcon tube (usually makes sense to prepare 4-5 of these)
- Wrap the falcon tube with aluminum foil so that it is minimally exposed to sunlight
- Store 10X working solutions at 4°C (they should be good for up to 6 months)
- Can also store one 1000X stock at 4°C for convenience (if planning to assay frequently)

Materials

PBS by Contributed by users

Resazurin sodium salt R7017 by Millipore Sigma

alamarBlue™ Cell Viability Reagent DAL1025 by Thermo Fisher Scientific

Protocol

Step 1.

Add 10X dye for each well using a multichannel pipettor (so, for 200 ul you will be adding 20 ul of the 10X solution)

Step 2.

Continue culturing the cells the same way for 24 hours

Step 3.

Use a plate reader to measure the absorbance at both 570 nm and 595 nm

EQUIPMENT
Equipment brand:
BIO-RAD
SKU:
1681130
Specifications:

Step 4.

Subtract your 595 nm measurement from the 570 nm for each well

Step 5.
Then (optionally) subtract your blank media measurement from your treatment measurements

Step 6.
The number you end up is directly proportional to the number of cells in the well.