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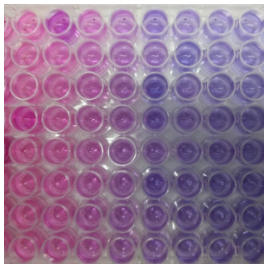
# Resazurin viability assay for human primary T cells in 96-well format



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**Protocol status:** Working
**We use this protocol and it's working**

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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

## Materials

MATERIALS

PBS

Resazurin sodium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**R7017

alamarBlue™ Cell Viability Reagent **Thermo Fisher Scientific Catalog #**DAL1025

## Troubleshooting

## 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)

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)

2 Continue culturing the cells the same way for 24 hours
24:00:00

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

Equipment

| | |
|---|---|
| new equipment | NAME |
| BIO-RAD | BRAND |
| 1681130 | SKU |

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

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

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