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 We use this protocol and it's working

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

👤 In 6 collections

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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

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🔗 PBS **Contributed by users**

🔗 Resazurin sodium salt **Millipore Sigma Catalog #R7017**

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

BEFORE START INSTRUCTIONS

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 μM and our goal is to prepare a 10X solution for use in cell culture (e.g. for 200 μl of media/cells, we will add 20 μl 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 μM):

- Add 500 μl 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 μl you will be adding 20 μl 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	
	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.