Cellevate

Cell viability*

Cell viability can be measured using the reagent alamarBlue® from Molecular Probes (single, ready-to-use solution).

1. Determine appropriate cell plating density as described in the manufacturers protocol.
   Note: Recomended cell numbers differ for each cell type but typical starting cell numbers are 4.000 for adherent cells and 5.000 - 10.000 for dissociated stem cells respectively.

2. Plate dissociated cells, see the protocol "seeding cells" for suggestion on procedure, seeding densities etc., in presence of test compounds or vehicle.

3. Add the alamarBlue® reagent as 10 % of the total sample volume (i.e., add 10 µL alamarBlue® reagent to 100 µL sample) of the and incubate at 37 oC for 1 - 4 hours, **protected from direct light**. Longer incubation times may be used for greater sensitivity without compromising cell health.

4. Transfer 100-200 µL of the content of each well into a fresh, clear plastic tissue culture plate.

5. Record results using fluorescence or absorbance as follows:
   a) **Fluorescence:** Read fluorescence using a fluorescence excitation wavelength of 540–570 nm (peak excitation is 570 nm). Read fluorescence emission at 580–610 nm (peak emission is 585 nm).

   b) **Absorbance:** Monitor the absorbance of alamarBlue® at 570 nm, using 600 nm as a reference wavelength (normalized to the 600 nm value).

   Note: Fluorescence mode measurements are more sensitive. When fluorescence instrumentation is unavailable, monitor the absorbance of alamarBlue® reagent. Assay plates or tubes can be wrapped in foil, stored at 4˚C, and read within 1–3 days without affecting the fluorescence or absorbance values.

6. **Optional:** Add 50 µL 3% SDS directly to 100 µL of cells in alamarBlue® reagent to stop the reaction.

*Suggested procedure, please adjust according to your experimental needs.

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General guidelines:

Be sure to include appropriate assay controls. To minimize experimental errors, we recommend making measurements from a minimum of 4–8 replicates of experimental and no-cell control samples.

You may need to determine the plating density and incubation time for the alamarBlue® assay for each cell type and use conditions such that the assay is in the linear range. (See manufacturers protocol).

If you plan to use longer incubation time (overnight), be sure to maintain sterile conditions during reagent addition and incubation to avoid microbial contaminants. Contaminated cultures will yield erroneous results as microbial contaminants also reduce alamarBlue® reagent.

Fetal bovine serum (FBS) and bovine serum albumin (BSA) cause some quenching of fluorescence. We recommend using the same serum concentration in controls to account for this quenching. Other media components, such as phenol red do not interfere with the assay.