Protocol Modification To Determine The Cytotoxic Potential Of Drugs Using Cell Viability Assays That Rely On The Reducing Property Of Viable Cells

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Abstract

In a recent article published in Nature’s Laboratoy Investigation journal, we demonstrated that drugs with anti-oxidant properties can interfere with cell viability measurements by assays that rely on the reducing property of viable cells, giving spurious results. The current manufacture recommended protocols of the assays assume that the assay reagent is almost entirely reduced intracellularly in viable cells. The potential for direct reduction of the reagent substrate by the drug being tested is somewhat underestimated (with the test compound control stated to be 'optional' in the Cell Titer Blue protocol). Given that these assays are convenient and widely used, this can have significant implications with indigenous compounds/ drugs having anti-oxidant properties and being screened for their cytotoxic potential against cancer, running the risk of being discarded due to spurious results. The article also demonstrates that by incorporating simple modifications in the protocol, these spurious results can be avoided.

Here-in, the modified Cell Titer Blue assay protocol is discussed step-by-step. The specific modifications added to the manufacture recommended protocol are in bold font. The rest is as stated in the manufacture recommended protocol.

https://www.promega.com/-/media/files/resources/protocols/technical-bulletins/101/celltiter-blue-cell-viability-assay-protocol.pdf

The modifications can also be incorporated in other cell viability assays that rely on the reducing property of viable cells such as Alamar Blue assay, MTT assay etc.

Reagents

Cell Titer Blue reagent

Equipment

• 96 well tissue culture plates compatible with fluorometer (clear or solid bottom)

• multichannel pipettor

• reservoirs to hold the Cell Titer Blue Reagent

• fluorescence reader with excitation 530–570nm and emission 580–620nm filter pair

Procedure

1. Test Compound Control:

CRITICAL: Prior to performing the cytotoxicity assay with any new test compound, always set up triplicate wells containing the vehicle and the test compound, incubate for the time period (1-4 hours) planned for the cytotoxicity assay and record fluorescence at 560/590nm. Performing this step will confirm whether or not the test compound directly reduces (or interacts in any other way with) the reagent dye.

If it is found that the test compound interferes with the reagent chemistry, it is necessary to perform the modification stated in the cytotoxicity assay protocol below (steps 6-8)**.** If found that the test compound does not interfere in any way with the reagent chemistry and the fluorescence recording between the vehicle and the drug is essentially the same, then the modification stated in the assay protocol below can be omitted, and the manufacture recommended protocol can be followed.

(The current manufacture recommended protocol states that this test compound control step is optional, but based on the findings in the article, we recommend that this step be performed for every new drug tested.

If this step is not performed, then the modification in the assay protocol below must be performed always, to counter any potential unknown drug interaction with the reagent chemistry).

Other controls such as ‘No cell control’, ‘Untreated cell control’ and ‘Positive Control for Cytotoxicity’ can be set up along with the assay protocol, as stated in the manufacture recommended protocol

2. Set up 96-well assay plates containing cells in culture medium.
3. Add test compounds and vehicle controls to appropriate wells so the final volume is 100µl in each well.
4. Culture cells for the desired test exposure period.
5. Remove assay plates from 37°C incubator.

6. For adherent cell lines, gently suction the contents of the well by placing the suction tip at the bottom edge of the well with a low suction pressure. **(For non-adherent cell lines, centrifuge the cells at 1200rpm for 5 min before performing steps 6-8, and suction gently.)**

7. Add 100ul of Phosphate Buffered Saline (PBS) to wash the cells and suction the PBS in

the same manner.

8. Add 100uL of fresh media.

9. Add 20µl/well of Cell Titer-Blue Reagent and shake for 10 seconds.

10. Incubate using standard cell culture conditions for 1–4 hours.

11. Shake plate for 10 seconds and record fluorescence at 560/590nm.

Troubleshooting

Unexpectedly low fluorescence reading in non-adherent cell lines.

Solution: Ensure that the cells have settled at the bottom of the well after centrifugation and suction very gently with low suction pressure, with the tip at the bottom edge of the well, to prevent suctioning the cells.

Anticipated Results

as in the reference article.

References

Shenoy N, et al. Drugs with anti-oxidant properties can interfere with cell viability measurements by assays that rely on the reducing property of viable cells. Lab Invest. 2017 Feb 27. doi: 10.1038/labinvest.2017.18.

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