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Update Notice: A Simple Microplate Assay for Reactive Oxygen Species Generation and Rapid Cellular Protein Normalization

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After official publication in Bio-protocol (https://bio-protocol.org/e3877), we noted some improvements in our protocol. The edits to be performed are the following:

1. The authors have improved background with a lower dye concentration in Step 7 in Figure 3 should read 35 µl rather than 50 µl.

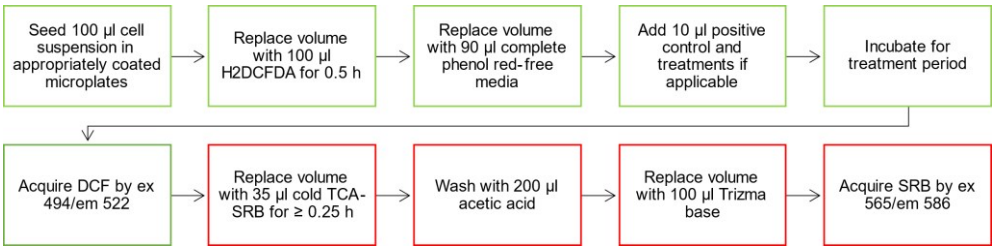

Figure 3. Protocol schematic. Cells are loaded with DCF-based probe, incubated for desired treatment period (e.g., 1 h or 24 h) before plate spectroscopy readout of DCF as an indication of ROS generation. To normalize DCF signal to cell population per well, a simultaneous acidic protein precipitation stage and cellular protein dye stage is performed before solubilization and spectroscopic readout of SRB. The TCA-SRB assay can be utilized to obtain relative cell populations for growth inhibition, cytotoxicity or to normalize other cell-based assay readouts.

2. In the Procedure Section, the volume of TCA-SRB in Step C2 is 35 µl. The Step C3 edits are the following: **Empty plate into an appropriate corrosives waste container and wash with 200 µl 1% acetic acid. A second wash may be required if the background is higher than expected, particularly with automated pipetting devices that cannot completely remove liquid from the corner of the well.**
3. In the Notes Section, Protocol is not efficient for suspension cells after further testing.
   "This protocol is intended for adherent cells; for more effective fixation of loosely adherent cells or suspension cells up to 50% w/v TCA may be utilized (Kim et al., 1996)." Is replaced with "**This protocol is intended for adherent cell types.**"

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