Protocol: Fivephoton Cell Viability and Proliferation Kit

Part: FCVK

Cell Number Determination

1. Prepare cell suspensions that are 5000 cells/100 µl for each well in a 96-well dish. Prepare one plate as a standard-control to compare the readout of the assay for a known number of cells/well to a plate with the same number of cells/well that are experimentally treated.

2. Inoculate cell suspension in a 96-well plate and culture in a humidified incubator overnight (e.g., at 37°C, 5% CO_2). .

3. Add 10 µl of assay solution for each well of the plate.
4. *Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.*

5. Incubate the plate for 1 - 4 hours in the incubator.

6. Measure the absorbance at 450 nm using an absorbance microplate reader.
7. *To measure the absorbance later within a 24hr period, add 10 µl of 1% w/v SDS or 0.1 M HCl to each well, cover the plate and store it protected from light at room temperature.*

Cell Proliferation and Cytotoxicity Assay

1. Dispense 100 µl of cell suspension (5000 cells/well) in a 96-well plate. Pre-incubate the plate overnight in a humidified incubator (e.g., at 37°C, 5% CO_2).

2. Add 10 µl of various concentrations of substances to be tested to the plate.

3. Incubate the plate for an appropriate length of time (e.g., 6, 12, 24 or 48 hours) in the incubator.

4. Add 10 µl of Fivephoton Cell Viability and Proliferation solution to each well of the plate.

5. Incubate the plate for 1 - 4 hours in the incubator.

6. Measure the absorbance at 450 nm using a microplate reader.

7. *To measure the absorbance later, add 10 µl of 1% w/v SDS or 0.1 M HCl to each well, cover the plate and store it from light at room temperature. No absorbance change should be observed for 24 hr*