

## EZQuant™ Cell Quantifying Kit Protocol

### Cell Count Protocol

1. Inoculate cell suspension (100 µl/well) in a 96-well plate. Pre-incubate the plate in a humidified incubator (e.g., at 37° C, 5% CO<sub>2</sub>).
2. Add 10 µl of the EZQuant™ solution to each well of the plate.  
*Be careful not to introduce bubbles to the wells, since they interfere with the O.D. reading.*
3. Incubate the plate for 1 - 4 hours in the incubator.
4. Measure the absorbance at 450 nm using a microplate reader.  
*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 with protection from light at room temperature. No absorbance change should be observed for 24 hours.*

### Cell Proliferation/Cytotoxicity Protocol

1. Dispense 100 µl of cell suspension (5000 cells/well) in a 96-well plate. Pre-incubate the plate for 24 hours in a humidified incubator (e.g., at 37° C, 5% CO<sub>2</sub>).
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 EZQuant™ solution to each well of the plate.  
*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 a microplate reader.  
*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 with protection from light at room temperature. No absorbance change should be observed for 24 hours.*

