

Protocol for CCK8 (**Cell Counting kit – 8**)

1. The cells is washed with PBS and trypsinized prior to resuspension in fresh complete medium in a 1.5 mL microcentrifuge tube.then cell count.
2. Dispense 100 μ l of cell suspension (2500 cells/well) in a 96-well plate.
3. Pre-incubate the plates for 12/24/48/72 hours in a humidified incubator (e.g., at 37 $^{\circ}$ C, 5% CO₂).
- 4.In 12th/24th/48th/72th hour, dilute 10 μ L CCK-8 solution to 100 μ Ladd 100 μ l diluted CCK-8 solution to each well of the plate. Be careful not to introduce bubbles to the wells.
5. Incubate the plate for 2 hours in the incubator.
6. Measure the absorbance at 450 nm using a microplate reader.