14. Protocol for CCK8 (**Cell Counting kit − 8**)

·Material

PBS
1.5 mL microcentrifuge tube
96-well plate
humidified incubator
CCK-8 solution
microplate reader

·Steps

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 µl 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 (at 37°C, 5% CO2).
④ In 12th/24th/48th/72th hour, dilute 10 µL CCK-8 solution to 100 µLadd 100 µl 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

·Note

① After joining CCK-8 Solution, test it after incubation of 2 h at 37 °C. If the time is not enough to add 1% SDS solution to each hole, room temperature light preservation, 24 h internal inspection, absorbent value will not be affected (the volume of adding 1% SDS solution is the same as the volume of adding CCK-8 Solution).
② CCK-8 Solution itself is low and does not affect cell growth. Therefore, cells treated with CCK-8 Solution can be discarded and then added to cell culture fluid for continuous culture.
③ If the measured absorbent value is very low we can increase the number of cells or extend the incubation time after joining CCK-8 Solution.