Protocol for cell culture and subculture

1. Culture in a humidified incubator at 37℃, 5% CO_2.

2. Check the color medium, healthy usually leaves medium slightly orange; observe the cells under phase microscope.

3. If the cells are not very confluent, remove the growth medium and replace with fresh complete medium.

4. If the cells are at least 70 %~ 80% confluence, subculture them.

5. Subculturing cells (60 mm×15 mm cell culture plate):

(1) Aspirate the growth medium from the cells.and wash the cells with PBS.

(2) Aspirate the PBS, then add 600μL trypsin EDTA solution.

(3) Incubation for several minutes at 37℃ and check the culture with inverted microscope to be sure that the cells are rounded up which indicates they are detached from the surface.

(4) Add 2 mL fresh complete medium,then pipet all the adherent cells into cell suspension and pipet up and down.

(5) Transfer the cell suspension into a 15 mL centrifuge tube and centrifuge at 1200 rpm for 5 minutes.

(6) Aspirate the filtrate ,then add 1mL PBS and resuspend the cell.

(7) Place 5 ml of fresh medium to each new culture .Add equal volume of cell suspension to each of the fresh plates that have been appropriately labelled.

(8) Culture in a humidified incubator at 37℃, 5% CO_2.