Protocol for Passaging Adherent Cells

1) Discard the spent cell culture medium from the culture vessel.
2) Wash cells with PBS solution without calcium and magnesium (approximately 2 mL per 10 cm^2 culture surface area). Gently add wash solution to the side of the vessel opposite the attached cell layer to avoid disturbing the cell layer, and rock the vessel back and forth several times.

   [Note]: The wash step will remove serum, calcium, and magnesium that would inhibit the effect of the dissociation reagent such as trypsin.
3) Remove and discard the wash solution from the culture vessel.
4) Add the pre-warmed trypsin to the side of the flask, the volume of the trypsin could cover the cell layer (approximately 0.5 mL per 10 cm^2). Gently shake the vessel to cover the cell layer completely.
5) Incubate the culture vessel at room temperature or 37℃ for approximately 2 minutes.

   [Note]: That the actual incubation time varies depending on the cell type.
6) Observe the cells under the microscope for detachment. If cells are less than 90% detached, increase the incubation time and check the dissociation every 30 seconds. You may also pat the vessel to expedite cell detachment.
7) When 90% of the cells detached, tilt the vessel for a minimal length of time to allow the cells to drain. And add the equivalent of 2 volumes (twice the volume of trpsin) of pre-warmed complete growth medium. Disperse the cells by pipetting the medium over the cell layer surface for several times.
8) Transfer the cell suspension to a 15 mL centrifuge tube and then centrifuge at 500 × g for 5 to 10 minutes.

   [Note]: That the centrifuge speed and time vary depending on the cell type.
9) Resuspend the cell pellet in a minimal volume of pre-warmed complete growth medium and take a sample for counting the number of cells.
10) Determine the total number of cells and percent viability and add growth medium to the cells to achieve the desired cell concentration.

[Note]: It is recommended to use the Countess Automated Cell Counter to determine the total number of cells and percent viability. Using the same amount of sample with the hemacytometer, the Countess Automated Cell Counter takes less than a minute per sample for a typical cell count and is compatible with a wide variety of eukaryotic cells.

11) Dilute cell suspension to the seeding density recommended for the cell line and pipet the appropriate volume into new cell culture vessels, then place the cells to the incubator.

[Note]: If the culture flasks are used, loosen the caps before placing the cells to the incubator to allow proper gas exchange unless vented flasks with gas-permeable caps are used.