Sub-culturing (splitting) protocol for HEK293 cells:

Materials:

• Culture vessel containing the cells.

• Tissue culture–treated plates.

• Complete growth medium, prewarmed to 37C.

• Phosphate-Buffered Saline (PBS), prewarmed to 37C.

• Dissociation reagent such as trypsin, prewarmed to 37C.

Protocol:

1. Remove and discard the spent cell culture medium from the culture vessel, using a vacuum pump.

2. Wash cells using PBS (approximately 5 mL per 10 cm2 culture surface area). Gently add the wash solution to the side of the vessel and rock the vessel back and forth several times.

Note: The wash step removes any traces of serum, calcium, and magnesium that would inhibit the action of the dissociation reagent.

3. Remove and discard the wash solution from the culture vessel.

4. Add 2 mL trypsin enzyme to the center of the plate. Gently rock the container to get complete coverage of the cell layer.

5. Incubate the culture vessel at 37° C for approximately 2 minutes.

6. Observe the cells under the microscope for detachment. When ≥90% of the cells have detached, return the plate into the hood and add 8 mL of complete growth medium to the plate.

7. Disperse the medium by pipetting over the cell layer surface several times. Move all 10 mL of cells to a 15 mL falcon and continue to pipet to avoid spores.

11. Transfer the appropriate volume of cells suspension and complete growth medium into new cell culture vessels in order to get to the desired seeding density.

12. Label the plate with the following information and return the cells to the incubator.

Cell line, date, number of passage and dilution (1:5, 1:8, 1:10 etc.)