Protocol for lipo 3000 transfection with Lipo fectamine $^{\text{TM}}$ 3000 Reagent

- 1. Seed cells to be 70–90% confluent at transfection.
- 2. Dilute 3.75-7.5μL LipofectamineTM 3000 Reagent in 250μL Opti-MEMTM Medium (2 tubes).
- 3. Prepare master mix of DNA by diluting DNA in Opti-MEMTM Medium, then add $P3000^{TM}$ Reagent Mix well.
- 4. Add Diluted DNA to each tube of Diluted Lipofectamine™ 3000 Reagent (1:1 ratio).
- 5. Incubate for 10–15 minutes at room temperature.
- 6. Add DNA-lipid complex to cells of the 6-well plate.
- 7. Incubate cells for 2–4 days at 37°C. Then, visualize/analyze transfected cells.