## Protocol for Lipofectamine® 2000 Transfection

- 1. Seed cells to be 70–90% confluent at transfection.
- 2. Dilute 5.0–12.5  $\mu L$  Lipofectamine® Reagent in 250  $\mu L$  Opti-MEM® Medium.
- 3. Dilute 2500 ng DNA in Opti-MEM® Medium.
- 4. Add diluted DNA to diluted Lipofectamine® 2000 Reagent (1:1 ratio).
- 5. Incubate for 20 minutes at room temperature.
- 6. Add DNA-lipid complex to a well of the 6-well plate.
- 7. Incubate cells for 12 h at 37  $^{\circ}$ C, 5% CO<sub>2</sub>, then Aspirate the growth medium from the cells and add fresh complete medium.
- 8. Incubate cells to 36 h at 37  $^{\circ}$ C, 5% CO<sub>2</sub>, then Visualize/analyze transfected cells.