# FuGENE 6 Quick Protocol

## Preparing the FuGENE 6® Transfection Reagent

1. Before use, allow the vial of FuGENE® 6 Transfection Reagent to reach room temperature
2. Mix by inverting or vortexing briefly. If a precipitate is visible, briefly warm at 37 degrees C then cool to room temperature

## General Transfection Protocol

1. To a sterile tube or U- or V-bottom plate add room temperature medium so that the final volume after adding the FuGENE 6® Reagent (in step 2) and DNA (in step 4) is 100µl.
2. For a 3:1 FuGENE® 6 Transfection Reagent to DNA ratio, add 6µl of FuGENE® 6 Transfection Reagent directly to medium, and mix immediately. For other ratios, consult table below.

| Tube Label | Medium Final (ul) | FuGENE® 6 Reagent (ul) | DNA (ug) |
|---|---|---|---|
| 2:1 | 100 | 4 | 2 |
| 3:1 | 100 | 6 | 2 |
| 4:1 | 100 | 8 | 2 |
| 6:1 | 100 | 12 | 2 |

3. Incubate the FuGENE® 6 / Medium mixture for 5 minutes at room temperature.
4. Add 2ug of DNA to the FuGENE® 6/Medium mixture (0.2-1.0 ug/ul) to a final volume of 100ul total. Vortex immediately,
5. Incubate complex at Room temperature for a minimum of 15 minutes. Up to 30 minutes
6. Add 2-10ul of the FuGENE® 6 Transfection Reagent/DNA mixture per well to a 96-well plate containing 100µl of cells in growth medium. Mix by pipetting or using a plate shaker. Return cells to the incubator for 24–72 hours.
7. Measure transfection efficiency using an assay appropriate for the reporter gene. For transient transfection, cells are typically assayed 24–72 hours after transfection.
8. See additional protocol information in Technical Manual available on www.fugene.com
9. For additional support please visit us at www.fugene.com