FuGENE 4K Quick Protocol

Preparing for Transfection

1. Seed cells to be 50-90% confluent at time of transfection
2. Before use, allow the vial of FuGENE® 4K Transfection Reagent to reach room temperature
3. 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 (transfection mix enough to transfect one 35mm dish)

1. To a sterile tube or U- or V-bottom plate add room temperature medium to so that the final volume after adding FuGENE 4K® & DNA in Step 2 & 3 is 100µl total volume.
2. Add 2µg of plasmid DNA (0.2–1µg/µl) to prewarmed media and vortex.
3. For a 3:1 FuGENE® 4K Transfection Reagent:DNA ratio, add 6µl of FuGENE® 4K Reagent directly to medium, and mix immediately. For other ratios, consult Table 1.

Table 1. Volumes of FuGENE® 4K Various FuGENE 4K:DNA Ratios.

	Ratio of FuGENE® 4K to DNA					
	5.5:1	5:1	4.5:1	4:1	3.5:1	3:1
Medium to a final volume of	100µl	100µl	100µl	100µl	100µl	100µl
DNA amount	2µg	2µg	2µg	2µg	2µg	2µg
Volume of FuGENE 4K	11ul	10ul	9ul	8ul	7ul	6ul

4. Incubate the FuGENE® 4K Transfection Reagent/DNA mixture for 5-15 minutes at room temperature.
5. Add transfection Reagent/DNA mixture to 35mm dish containing cells in growth medium. Mix by pipetting or using a plate shaker. Return cells to the incubator for 24–48 hours.
6. Measure transfection efficiency using an assay appropriate for the reporter gene. For transient transfection, cells are typically assayed 24–48 hours after transfection.
7. See additional protocol information in Technical Manual available on www.fugene.com
8. For additional support please contact us at www.fugene.com