User Guide: FuGENE® 4K Transfection Reagent

For transient and stable transfection of eukaryotic cells with DNA

For life science research only

Cat. No 4K-1000 1ml

1. Product Overview

Application:

- Gene function/over expression
- Virus production
- Protein/Antibody production
- Stable cell line generation
- CRISPR

Benefits:

- High transfection efficiency in many common and difficult-to-transfect cell lines.
- Low cytotoxicity; reduces confounding variables in your experiment
- Functions well with cells in serum-containing medium; eliminates the need to exchange media
- Suitable for transient and stable-transfection
- Requires minimal optimization.

Formulation

FuGENE® 4K Transfection Reagent is a 100% synthetic, proprietary blend of lipids and other components supplied in 70% ethanol, 0.1um sterile-filtered, and packaged in glass-vials. It does not contain any ingredients of human or animal origin.

Shelf-life and Storage

- Shelf-life: at least 2 years from the date of manufacture when properly stored.
- Storage: In original glass vial, securely capped, protected from light, and between +2°C and +22°C

Important: If stored below room temperature, bring the reagent vial to room temperature and mix prior to use (vortex 1-2 s). If precipitate is visible, briefly heat the vial to 37°C, vortex, and allow to cool to room temperature before use.

Special Handling

Do not aliquot FuGENE® 4K from the original glass vials. Chemical residues in plastic tubes may decrease activity of the reagent. Prevent FuGENE® 4K from contacting the plastic walls of the tube by pipetting the reagent directly into the serum-free medium with DNA.

Note: An empty 24-well cell culture plate can be used as a rack for the FuGENE® 4K vial.

Quality control - *Activity and Cytotoxicity analysis*

FuGENE® 4K, at varying volumes, is combined with GFP-encoding plasmid DNA, and used to transfect A549, CHOK1, and HEK293 cells (at 50–90% confluence). 24 hours after transfection, GFP expression and propidium iodide staining are measured by flow cytometry to ensure the reagent meets internal activity and cytotoxicity standards for release.

2. Protocol

Preparation

1. 16 – 24 hours before transfection, plate cells in serum-containing growth medium so that the culture is 50-90% confluent at the time of transfection. Refer to Table 1 for recommended cell numbers by culture vessel size.
2. Before use, allow the vial of FuGENE® 4K and serum-free medium to reach room temperature.
3. Mix FuGENE® 4K by inverting or vortexing the vial for 1 – 2 seconds. If precipitate is visible, warm at 37°C then cool to room temperature and vortex.

Transfection

The following procedure is scaled to one well of a 6-well plate (or 35mm dish) at the recommended starting ratio of 4:1 (4µl of reagent: 1µg DNA). Please refer to Table 1 below for scaling to other vessel sizes. It is important to note that the reagent and DNA amount listed below is only a starting point and may need to be adjusted per cell line or application. Please see the Optimization section below for recommended reagent and DNA amounts to test initially with your cells and plasmid DNA.

1. Add a volume of **serum-free** medium (e.g. DMEM without serum or antibiotics) into a sterile microcentrifuge tube so the final volume after DNA and reagent addition (steps 2. and 3.) is 100ul.
2. Add 2 µg of plasmid DNA (0.2 – 1 µg/µl) to the reaction tube containing the medium. Mix well.
3. Pipet 8ul of FuGENE® 4K reagent directly into the diluted DNA in the reaction tube. Mix well and stand at room temperature for 5-15 minutes.
4. Add the contents of the reaction tube to 1 well of a 6-well plate containing cells in growth medium. Mix by horizontal motion. Note: for smaller wells, mix by pipetting up-and-down.
5. Incubate transfected cells at 37°C for 24-48 hours, then analyze.

Table 1: Transfection Parameters Scaled to Various Culture Vessel Sizes

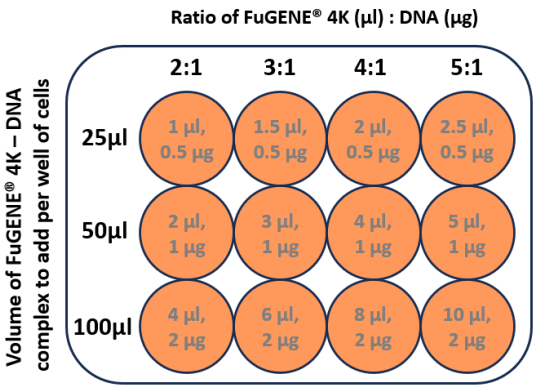
Culture vessel	Volume (ml) of Growth Medium per well	Cell Plating Density (number of cells per well)	Volume (µl) of Reagent-DNA Complex to add per well	DNA (µg) per well	FuGENE® 4K (µl) per well (4:1 ratio)
96-well plate	0.1	10,000 - 30,000	5	0.1	0.4
24-well plate	0.5	50,000 - 150,000	25	0.5	2
12-well plate	1	100,000 - 300,000	50	1	4
6-well plate / 35mm dish	2	200,000 - 600,000	100	2	8
6cm dish	5	500,000 - 1,500,000	250	5	20
10cm dish	12	1,200,000 - 3,600,000	600	12	48

3. Optimization

- **Reagent and DNA amount:** The optimal amount of FuGENE® 4K and plasmid DNA for transfection may vary between cell lines, cell culture conditions, and application. To determine the amount of FuGENE® 4K and DNA that is optimal for your system, perform the recommended *Optimization Experiment* below.
- **Other parameters:** if sufficient transfection is not achieved after optimizing the reagent and DNA amount, it may be necessary to optimize the following; cell plating density, incubation time for the FuGENE® 4K – DNA complexation step, time of transfection relative to cell plating, serum-free media types for FuGENE® 4K – DNA complexation, time of assay relative to transfection.

➢ *Optimization Experiment*:

1. Add 188µl, 184µl, 180µl, or 176µl serum-free medium to 4 sterile tubes labeled 2:1, 3:1, 4:1, or 5:1, respectively.
2. Add 4 µl of 1 µg/µl plasmid DNA (4 µg) to medium in each tube, mix.
3. Add 8µl, 12µl, 16µl, or 20µl of FuGENE® 4K to the tubes labeled 2:1, 3:1, 4:1, or 5:1, respectively. Mix well after each addition of reagent, then stand the 4 tubes at room temperature for 5-15 minutes**.**
4. Transfer 25µl, 50µl, or 100µl from each tube to individual wells of a 12-well plate of cells (50-90% confluent). Gently mix the plate.
5. Incubate cells at 37°C for 24 – 48 hours before analyzing transfection efficiency.



Final amount of FuGENE® 4K and DNA per well in gray

4. Notes and Considerations

- **Reducing culture confluence**: If less confluence is needed at the time of your experiment (>24 hours after transfection), cells can be sub-cultured the day after they are transfected. Alternatively, seed fewer cells so the culture is <50% confluent at the time of transfection and reduce the amount of reagent-DNA complex added.
- **Cell culture conditions**: Minimize variance in transfection efficiency by using cells that are maintained by regular subculturing, plated at a consistent density for transfection, proliferating well (log-growth phase) when transfected, and *Mycoplasma*-free. Cell lines can lose their transfection competence after prolonged subculturing. Restart the cell line from a frozen stock if a loss in transfection efficiency is observed with an old culture.
- **Plasmid DNA**: Stock DNA should be 0.2 µg/µl – 1 µg/µl in sterile TE buffer or water with A_{260}/A_{280} ~1.8. Plasmid DNA prepared by commercially available Miniprep kits is suitable to transfect many cell lines. However, for cells sensitive to endotoxins, DNA of greater purity may be required.
- **Other media additives**: Antimicrobial agents (e.g., antibiotics and fungicides), that are commonly included in cell-culture media, may adversely affect the transfection efficiency. If possible, exclude additives for initial experiments. Once high-efficiency conditions have been established, these components can be added back while monitoring your transfection results.
- **Co-transfection**: when transfecting more than one plasmid, it is important that the reagent:DNA ratio is maintained for the total DNA input. For example, to transfect 2 plasmids using a 4:1 ratio, combine 0.5 µg of each plasmid in serum-free medium (total DNA is 1 µg), then add 4 µl of reagent. The molar ratio of the plasmids can be altered to adjust their relative expression, but the reagent:DNA ratio should not change.
- **Controls**: transfect cells with non-coding DNA, add reagent only (-DNA), or no treatment
- **Cytotoxicity**: Uptake of DNA can be toxic to cells. If excessive cytotoxicity is observed after transfecting non-coding DNA (i.e. toxicity is not caused by the expressed transgene), make one or more of the following changes to the protocol; reduce the volume of FuGENE® 4K – DNA complex added, increase the number of cells plated for transfection, or change medium 4 – 6 hours after transfection

Contact and Technical Support:

For additional troubleshooting techniques or other questions please contact us directly at contact@fugene.com

Notice to Purchaser:
Purchaser represents and warrants that it will use FuGENE® 4K Transfection Reagent purely for research purposes. Transfected cells, materials produced, and any data derived from the use of FuGENE® 4K Transfection Reagent, may be used only for the internal research of Purchaser whether Purchaser is a "for-profit" or a "not-for-profit" organization. Under no circumstances may FuGENE® 4K Transfection Reagent be used by Purchaser or any third party for a commercial purpose unless Purchaser has negotiated a license for commercial use with Fugent, LLC (contact information: contact@fugene.com). For purposes of the foregoing sentence, "commercial purpose" shall mean use of FuGENE® 4K Transfection Reagent for profit or commercial gain. By using FuGENE® 4K Transfection Reagent, Purchaser agrees to be bound by the above terms. If Purchaser wishes not to be bound by these terms, Purchaser agrees to return the FuGENE® 4K Transfection Reagent to Fugent LLC. for a full refund.

Trademarks:
FuGENE® is a registered trademark of Fugent LLC.