FuGENE 6
Transfection Reagent

For the transient and stable transfection of animal cells

Cat. No. 11 815 091 001 0.4 ml (120 transfections)
Cat. No. 11 814 443 001 1 ml (300 transfections)
Cat. No. 11 815 075 001 Multi-pack 5 × 1 ml (1,500 transfections)
Cat. No. 11 988 387 001 Mega-pack 5 × 1 ml (1,500 transfections)
Cat. No. 11 988 484 001 Custom pack
Inquire (10-ml or 50-ml glass vials)

*Available only in the U.S.

Note: This is an abbreviated package insert that is intended for use by researchers who are experienced with transfection. For researchers who are starting transfection experiments for the first time, detailed instructions are available at www.roche-applied-science.com/fugene/instructions

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Product Characteristics

**Formulation**

FuGENE 6 Transfection Reagent is a proprietary blend of lipids and other components supplied in 80% ethanol, sterile-filtered, and packaged in glass vials.

**Storage and stability**

**FuGENE 6 Reagent is shipped at room temperature.**

FuGENE 6 Transfection Reagent is stabilized for extended storage at +2 to +8°C through the expiration date printed on the label (two years from the date of manufacture) when very tightly closed. Always mix FuGENE 6 Reagent prior to use (vortex for one second or use inversion). FuGENE 6 Reagent may also be stored at –15 to –25°C. Regardless of the storage temperature used, be sure to allow the FuGENE 6 Reagent to come to room temperature prior to use.

**Special handling**

Do not aliquot FuGENE 6 Reagent from the original glass vials. Chemical residues in plastic vials can significantly decrease the biological activity of the reagent. Minimize the contact of undiluted FuGENE 6 Reagent with plastic surfaces. Always dilute the reagent by pipetting directly into serum-free medium. Do not allow the FuGENE 6 Reagent to contact the plastic walls of the tube containing the serum-free medium during the dilution step.

**Note:** FuGENE 6 Transfection Reagent remains fully functional even after repeated vial openings (at least six times over a three-month period) as long as the vials are tightly recapped and stored at +2 to +8°C between uses.

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Quality control

**Functional analysis**

Three microliters of FuGENE 6 Transfection Reagent is combined with 1–2 μg of reporter-gene vector DNA, and used to transfect COS-1 cells (in a monolayer [50–80% confluent]) in the presence of 10% fetal bovine serum (FBS). Following transfection, the percentage of transfected cells is analyzed. Typically, 50–70% of COS-1 cells express reporter-gene protein.

**Cytotoxicity analysis**

COS-1 cells that are continuously exposed to FuGENE 6 Reagent for 26 hours, with or without DNA, in the presence of serum, and without a change of medium, are >90% viable by flow-cytometric analysis based on propidium-iodide staining.

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Background information

**Application**

FuGENE 6 Transfection Reagent is a multi-component reagent that forms a complex with DNA, then transports it into animal cells. Benefits of FuGENE 6 Reagent include:

- High transfection efficiency in many common cell types, including HeLa, NIH 3T3, COS-1, COS-7, and CHO-K1. For a list of >600 cell types that have been successfully transfected with FuGENE 6 Reagent, visit www.roche-applied-science.com/fugene/instructions
- Demonstrates virtually no cytotoxicity, allowing you to work with fewer cells, and eliminates the requirement to change media after the addition of transfection complex
- Suitable for transient and stable transfection
- Functions exceptionally well in the presence or absence of serum; eliminates the need to change media
- Requires minimal optimization.

† Each of the five vials is individually packaged.
‡ The five vials are packaged together in one box with one pack insert.
It is critical to accurately determine the plasmid DNA concentration using 260-nm absorption. DNA content must be determined by 260-nm absorption (estimates of DNA content based on the intensity of gel bands are not sufficiently accurate). Determine the DNA purity using a 260 nm/280 nm ratio; the ratio should be 1.8. Transfection-grade plasmid preparations can be isolated using the Genopure Plasmid Midi Kit (Cat. No. 03 143 42 001), Genopure Plasmid Maxi Kit (Cat. No. 03 143 42 001), or High Pure Plasmid Isolation Kit (Cat. No. 11 754 777 001 and 11 754 785 001, mini preps).

Cell-culture conditions

Minimize both intra- and interexperimental variance in transfection efficiency by using cells that are regularly passaged, proliferating well (best when in a log-growth phase), and plated at a consistent density.

Other media additives

In some cell types, antimicrobial agents (e.g., antibiotics and fungicides) that are commonly included in cell-culture media may adversely affect the transfection efficiency of FuGENE 6 Transfection Reagent. Up to a 25% decrease in efficiency has been observed. 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.

Verification of vector function

Optimize transfection conditions with a known positive-control reporter-gene construct (see Mammalian Expression Vectors in “Related products” section) prior to transfecting cells with a new vector construct:

- Determine transfection efficiency with a reporter-gene assay (CAT, β-Gal, Luciferase, SEAP, or hGH [see “Related products” section]).
- Sequence across the flanking vector insert regions to verify the integrity of your new construct.

High protein-expression levels

High expression levels of certain intracellular proteins (e.g., GFP) may be cytotoxic to some cell types. Cell proliferation, toxicity, and cell death may be monitored using Roche Apoptosis and Cell Death products (visit www.roche-applied-science.com/sis/apoptosis for more information).

Incubation time

Incubate the cells for 4–72 hours. The length of incubation depends upon the transfected vector construct, the cell type being transfected, and the type of protein being expressed. After this incubation period, measure protein expression using an assay that is appropriate for your system.

### Procedures and Required Materials

#### Before you begin

**Additional required reagents and supplies**

<table>
<thead>
<tr>
<th>Sterile, serum-free culture medium without additives or supplements (optional): add 12.5 mM HEPES buffer to serum-free medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid DNA solution (between 0.02 µg/µl and 2.0 µg/µl) in sterile TE (Tris/EDTA) buffer or sterile water.</td>
</tr>
<tr>
<td>To prevent spillage, use a 24-well plate as a test tube rack for the FuGENE 6 Transfection Reagent.</td>
</tr>
</tbody>
</table>

#### Preparation of cells for transfection

**Adherent cells**

One day before the transfection experiment, trypsinize, adjust the cell concentration, and plate the cells in the chosen cell-culture vessel. For most cell types, plating 1–3 x 10^5 cells in a 35-mm culture dish in 2 ml of medium (or a six-well plate) overnight will achieve the desired density of 50–80% confluency. If using culture plates of a different size, adjust the starting volume of FuGENE 6 Reagent and the starting mass of DNA in proportion to the relative surface area (Table 1).

**Suspension cells**

Use freshly passaged cells at a concentration of 5 x 10^5/ml to 1 x 10^6/ml (2 ml in a 35-mm culture dish or six-well plate). Determine the cell number based on your needs and the cell type to be transfected.

#### Preparation of FuGENE 6 Reagent:DNA complex and transfection of cells

**Adherent and suspension cells in a 35-mm culture dish**

For initial optimization, use FuGENE 6 Reagent:DNA ratios of 3:1, 3:2, and 6:1 (µl, for FuGENE 6 Reagent, and µg for DNA, respectively). The preparation of the complex for a single well of a six-well plate, or a 35-mm culture dish, is described below. These ratios will function very well for commonly used adherent cells and suspension cells.

**Important:** The FuGENE 6 Reagent:DNA complex must be prepared in medium that does not contain serum, even if the cells are transfected in the presence of serum.

**Note:** For additional optimization tips, go to www.roche-applied-science.com/fugene/instructions

#### Ratio overview

Preparation of a complex that is sufficient for a 35-mm culture dish, or one well of a six-well plate, at three different ratios:

<table>
<thead>
<tr>
<th>Tube label</th>
<th>SFM (µl)</th>
<th>FuGENE 6 Reagent (µl)</th>
<th>DNA (µg)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3:1</td>
<td>97</td>
<td>3</td>
<td>1</td>
<td>Add the entire volume to each well of a six-well plate, or 5 µl to each well of a 96-well plate.</td>
</tr>
<tr>
<td>3:2</td>
<td>97</td>
<td>3</td>
<td>2</td>
<td>Additional suggested volumes for different containers are included in Table 1.</td>
</tr>
<tr>
<td>6:1</td>
<td>94</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
Co-transfection experiments

Suggestions

Co-transfection experiments with FuGENE 6 Reagent have been simultaneously performed using up to seven different plasmids. Be sure to maintain the same total reagent:total DNA ratio as that used for a single plasmid in your system. If it is necessary to increase the total amount of DNA, then increase the amount of transfection reagent in proportion to the amount of total µg DNA when performing co-transfection experiments.

IMPORTANT: Always use a volume of FuGENE 6 Reagent that is in excess of the total final mass of DNA.