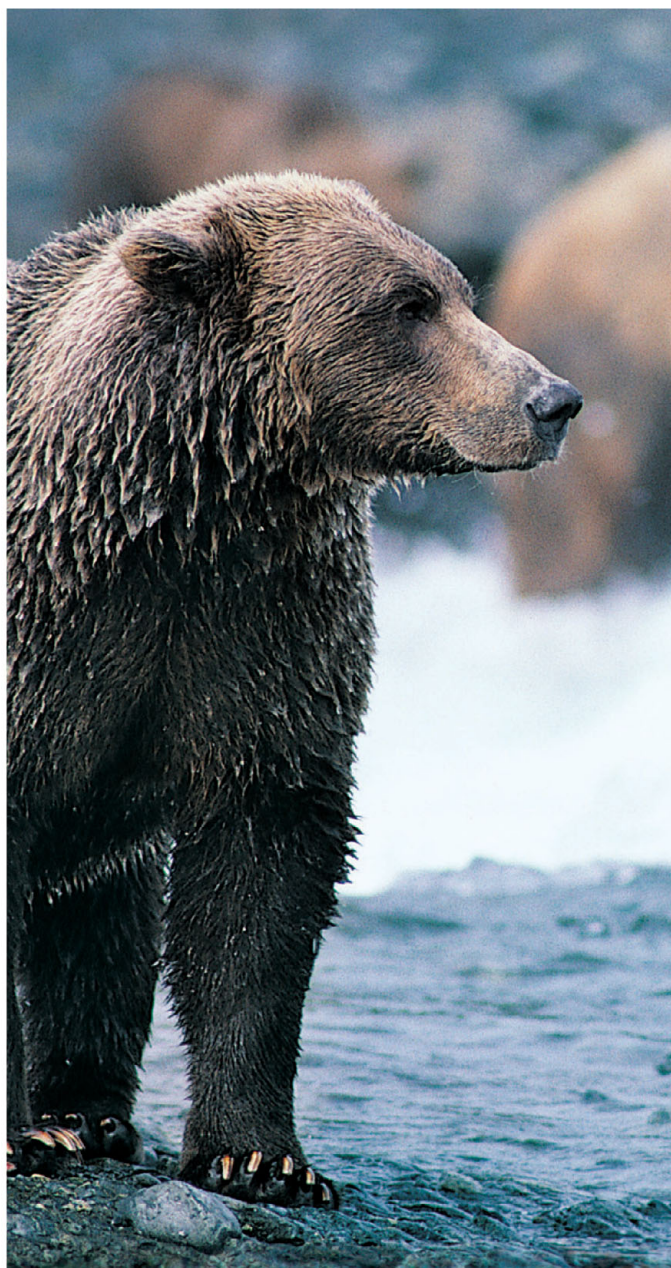


FuGENE[®] HD Transfection Reagent

Application Note No. 3 / November 2006



**Protocol for optimizing
transfection of adherent
cell lines**

Introduction

No single transfection protocol can be applied to all types of cells and experiments. Varying degrees of optimization may be required to obtain maximal transfection efficiency and protein expression with minimal cytotoxicity. The four parameters optimized with this protocol are:

- Ratio of transfection reagent to purified plasmid DNA.
- Time for transfection complex (transfection reagent and DNA) to form.

- Volume of transfection complex added to each well.
- Cell density at time of transfection.

This protocol tests these four parameters in a simple optimization experiment. Six different complexes are made using FuGENE® HD Transfection Reagent, and different amounts of the complexes are then added to the wells of a 96-well plate at three different time points.

Cell Growth Notes

- Roche recommends using freshly-obtained, low-passaged cell lines from ATCC®.
- Grow the cells in a medium recommended by provider.

- Passage the cells twice a week or as recommended to maintain log-phase growth (80-90% confluence). Dislodge the cells from the flasks by trypsinization or another appropriate method.
- Attempt to keep the passage level less than 50 to 60. If a change in the cells is observed, replace with fresh cells.

Day 1: Cell Plate Preparation

Choose a cell density (e.g., 5,000, 10,000, or 20,000 cells per well) and plate adherent cells on a 96-well plate the day before transfection.

This is the **cell plate**. Scale volume as needed for differently-sized plates or wells.

Day 2: Transfection Complex Plate Preparation and Cell Transfection

Materials

- Cell plate from Day 1, containing healthy cells.
- High quality, purified plasmid DNA (quantified).
- Diluent such as OptiMEM™ I Reduced Serum Medium or DMEM (for insect cells, use sterile, pure water).
- FuGENE® HD Transfection Reagent.
- New 96-well round-bottom tissue culture plate (preferably tissue culture treated).

Transfection Complex Plate Preparation

1. Dilute plasmid DNA by adding 16 µg DNA to 800 µl OptiMEM™ I Reduced Serum Medium or another serum-free medium to achieve a concentration of 20 µg/ml. Vortex briefly to mix.
2. Dispense 100 µl of the diluted DNA (2 µg plasmid/100 µl) into 7 wells of a new 96-well plate as shown in Table 1 (Wells B2 through H2). This is the **transfection complex plate**. Note that the top well (Table 1, Well A2) contains medium with no DNA.

Day 2: Transfection Complex Plate Preparation and Cell Transfection

3. Add the specified amounts of FuGENE® HD Transfection Reagent to Wells A2 through G2 (as shown in Table 1), to make six different ratios of reagent to DNA and one reagent control. Gently mix each well by pipetting up and down about 15 times immediately after adding reagent, then

change pipette tips and add reagent to the next tube. Note that the bottom well (Table 1, Well H2) does not receive any reagent.



Do not use siliconized pipette tips. Add reagent directly to the diluted DNA without touching the sides of the well.

Table 1: Transfection Complex Plate Layout

Row	Row Name	Column 2 Well Contents		
		Reagent	DNA	Medium
A	Reagent Control	8 μ l	0 μ g	100 μ l
B	8:2 ratio	8 μ l	2 μ g	100 μ l
C	7:2 ratio	7 μ l	2 μ g	100 μ l
D	6:2 ratio	6 μ l	2 μ g	100 μ l
E	5:2 ratio	5 μ l	2 μ g	100 μ l
F	4:2 ratio	4 μ l	2 μ g	100 μ l
G	3:2 ratio	3 μ l	2 μ g	100 μ l
H	DNA Control	0 μ l	2 μ g	100 μ l



Transfection

1. As soon as the last transfection complex has been formed, remove the cell plate from the incubator.
2. Using a multichannel pipetter, transfer various amounts of complex or control solutions from the **transfection complex plate** to the **cell plate**, as shown in Table 2 (see page 4); note that each row of wells on the transfection complex plate corresponds to the matching row on the cell plate. Add the complex directly into the medium and mix gently to ensure adequate mixing without disturbing cells.
 - a. Immediately add 8 μ l of complex to column 2, 6 μ l to column 3, and 4 μ l to column 4 (Table 2, green columns). Mix plate on platform shaker for 30 seconds. Return the **cell plate** to incubator for 10 to 15 minutes.
 - b. **Remove the cell plate from the incubator.** Add 8 μ l to column 5, 6 μ l to column 6, and 4 μ l to column 7 (Table 2, yellow columns). Shake for 30 seconds, and then return the **cell plate** to incubator for an additional 10 to 15 minutes.
 - c. **Remove the cell plate from the incubator.** Add 8 μ l to column 8, 6 μ l to column 9, and 4 μ l to column 10 (Table 2, blue columns). Shake for 30 seconds, and then return the **cell plate** to incubator.
3. Incubate the **cell plate** for 24, 48, or 72 hours depending upon the expressed gene.
4. Assay for protein. Prior to assaying the cells for expression of the transfected gene, visually inspect the cells.



The experiment may be repeated with different cell densities to determine optimal cell density for a given cell line, or different diluents for specific applications. Also, the amounts of added complex (8, 6, and 4 μ l) may be changed. For example, using 10, 5, and 2.5 μ l provides a broader initial range suitable for testing a new cell line or plasmid.

Table 2: Cell Plate Layout, Day 2 (Transfection)

		1	2	3	4	5	6	7	8	9	10	11	12
Reagent													
A Control	C		8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	C	C
B 8:2	C		8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	C	C
C 7:2	C		8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	C	C
D 6:2	C		8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	C	C
E 5:2	C		8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	C	C
F 4:2	C		8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	C	C
G 3:2	C		8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	C	C
H DNA Control	C		8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	8 µl	6 µl	4 µl	C	C
			No incubation			10 to 15 minutes of incubation			20 to 25 minutes of incubation				

Table Notes

- The listed volumes are the amount of prepared transfection complex to be added to each well.
- Wells labeled as “C” are wells containing cells to which no DNA, reagent, or complex are added. They serve as **cell controls**.

Ordering Information

Product	Pack Size	Cat. No.
FuGENE® HD Transfection Reagent	0.4 ml (up to 120 transfections)	04 709 691 001
	1.0 ml (up to 300 transfections)	04 709 705 001
	5 x 1 ml (up to 1,500 transfections)	04 709 713 001
	Trial-pack	04 883 560 001



To ensure the quality of cells to be transfected, Roche recommends using freshly-obtained, low-passaged cell lines from ATCC®. For more information, please visit and bookmark www.atcc.org

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