PROTOCOL

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Dharmacon™ DharmaFECT™ kb transfection reagent

The following is a general protocol for use of DharmaFECT kb transfection reagent to deliver plasmid into cultured mammalian cells. The examples given within the protocol are for 96-well plates, and Table 1 provides the transfection reagent volumes for additional plate types.

Reagents to be supplied by the user

Cells and appropriate growth medium. Appropriate antibiotic if researcher will perform selection.

General considerations

DNA quality requirements

DNA quality is critical for successful transfection. An A260/A280 ratio of 1.8 is recommended. Endotoxin-contaminated DNA may result in inefficient transfection and cause unacceptably high cellular toxicity.

Cell density

The recommended confluency for adherent cells on the day of transfection is 70–90%. Suspension cells should be in logarithmic growth phase.

Incubation time

Transient transgene expression takes place within 24-72 hours after transfection. The optimal incubation time depends on the cell type, promoter strength and expression product, and must be determined experimentally.

Transfection reagent and DNA amounts

The volume of transfection reagent used depends on the amount of DNA, transgene, and cells to be transfected. The ranges presented in the protocols below are starting amounts and should be further optimized for best results.

Choice of promoter

High transgene expression depends both on the transgene promoter and on the cell line used. Cytomegalovirus (CMV) promoter is commonly used for high gene expression in a variety of cell lines. Other promoters such as elongation factor 1 alpha (EF1a), ubiquitin C (UBC), phosphoglycerate kinase (PGK) and chicken beta actin hybrid (CAG) can also be used.

General protocol for transfection of adherent cells in 96-well format

Quantities and volumes should be scaled according to the number of wells to be transfected (Table 1).

1. In each well, seed cells in 100 µL of growth medium 24 hours prior to transfection at a density that will give a confluency of 70-90% for adherent cells on the day of transfection. Suspension cells should be in logarithmic growth phase at the time of transfection.
2. Dilute DNA in 10 µL per well of serum-free DMEM or other serum-free growth medium.

We recommend starting with 100-200 ng DNA per well. The amount of DNA used per well depends on transgene expression, assay to be used, and cell line. Amount of DNA should be optimized for each experiment.

3. Gently mix DharmaFECT kb transfection reagent by pipetting.
4. Dilute DharmaFECT kb reagent in 10 µL per well of serum-free DMEM or other serum-free growth medium.

Prepare immediately prior to transfection. User should optimize volume of DharmaFECT kb reagent used based on experimental system. Optimization of DharmaFECT kb reagent and DNA amounts will increase transfection efficiency specific for the cell line and transgene used.

5. Add 10 µL diluted DharmaFECT kb reagent to the diluted DNA for a total of 20 µL per well.
6. Incubate 10 minutes at room temperature.
7. Add 80 µL of growth medium to the complex and mix.

The transfection efficiency with DharmaFECT kb™ transfection reagent (Cat #T-2006-01) is equally high in the presence of serum. This is not the case with other transfection reagents.

8. Gently aspirate medium from cell culture plate.
9. Immediately dispense 100 µL of the transfection reagent/DNA mixture to each well.
10. Gently rock the plate to achieve even distribution of the complexes.
11. Incubate at 37 °C in a CO_2 incubator.
12. Begin to analyze transgene expression 24-72 hours later. For stable transfection, cells should be grown in selective medium.

If using DNA containing a fluorescent reporter gene, and you observe less than 90% of all cells expressing the transfected reporter gene, first try optimizing transfection conditions by varying cell confluency or density, volume of DharmaFECT kb reagent, and amount of DNA. If after optimization of transfection conditions less than 90% of cells express the reporter, antibiotic selection is recommended to eliminate those cells from the population that are not transfected.

Table 1. Scale-up ratios for transfection of adherent and suspension cells with DharmaFECT kb transfection reagent.

Tissue culture vessel	Growth area, cm^2/well	Volume of medium, mL	Adherent (suspension) cells to seed the day before transfection*	Amount of DNA		Volume of DharmaFECT kb, µL	
				µg**	µL***	Recommended	Range
96-well plate	0.3	0.1	0.5-1.2 × 10^4 (2.0 × 10^4)	0.2	10	0.6	0.4-1.0
48-well plate	0.7	0.25	1.0-3.0 × 10^4 (5.0 × 10^4)	0.5	25	1.5	0.8-2.2
24-well plate	2.0	0.5	2.0-6.0 × 10^4 (1.0 × 10^5)	1.0	50	3.0	2.0-5.0
12-well plate	4.0	1.0	0.4-1.2 × 10^5 (2.0 × 10^5)	2.0	100	6.0	3.9-9.0
6-well plate	9.5	2.0	0.8-2.4 × 10^5 (4.0 × 10^5)	4.0	200	9.0	6.0-12.0
60 mm plate	20	3.0	2.0-6.3 × 10^5 (1.0 × 10^6)	6.0	300	18.0	12.0-24.0

** These numbers were determined using HEK293T and U2OS cells. Actual values depend on the cell type.*
*** Amount of DNA and DharmaFECT kb transfection reagent used may require optimization.*
**** The volume of DNA should be 1/10 of the volume of the culture medium used for dilution of the DNA.*

Antibiotic selection

If the transfected vector confers resistance to Blasticidin S (Fisher Scientific, Cat #BP2647-25), puromycin (Fisher Scientific, Cat #BP2956-100), or another antibiotic, selection can be used to eliminate non-transfected cells. In order to generate stable cell lines, it is important to determine the minimum amount of antibiotic required to kill non-transfected cells. This can be done by conducting a dose response curve on the cell line of interest.

Protocol for selection using Blasticidin S or puromycin

If adding antibiotic for selection, use the appropriate concentration as determined based on the above dose response curve.

1. Add medium containing antibiotic 24 hours post-transfection to begin selection.
2. Cells can be harvested for transgene expression 24-72 hours after starting selection.

It is important to wait at least 24 hours after transfection before beginning selection.

3. If longer selection is required for cells to be confluent, replace selective medium approximately every 2-3 days.
4. Monitor the cells daily and observe the percentage of surviving cells. Cells surviving selection will be expressing the transgene.
5. If generating stable cell lines (optional), select and grow for 10-15 days.
6. Once non-transfected cells are eliminated and/or you have selected for stably transfected cell lines if desired, you can proceed to assay for transgene expression. RT-qPCR, western blot analysis or other appropriate functional assay can be used; compare treated samples to untreated, reporter alone, non-silencing control, or other controls as appropriate.

Frequently asked questions (FAQs)

Question	Answer
How can the DNA and DharmaFECT kb reagent be adjusted to improve transfection efficiency?	If reagent seems to be toxic to a particular cell line, try reducing. If low transfection efficiency is due to a suboptimal, try using different amounts of the transfection reagent while keeping the amount of DNA constant. If low transfection efficiency is due to a suboptimal amount of DNA, keep the constant while adjusting the amount of DNA used in the transfection.
Approximately how many transfections are available in each vial of DharmaFECT kb reagent?	The number of transfections that can be performed with each vial of DharmaFECT kb reagent will vary depending on the tissue culture plate used and the cell type. For example, the 1 mL vial of DharmaFECT kb will be sufficient for ~ 330 transfections in the 24-well plate format.

If you have any questions, contact

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