For research use only. Not intended for any
animal or human therapeutic or diagnostic use.

ATGCell

DNAFect® LT

For transient and stable transfection of mammalian cells

Catalogue Number: DF02-05, Size: 0.5 ml
Catalogue Number: DF02-10, Size: 1.0 ml
Catalogue Number: DF02-50, Size: 5.0 ml
Catalogue Number: DF02-100, Size: 10.0 ml

Product Summary

DNAFect® LT is an efficient and versatile reagent for gene delivery that can be used for *in vitro* transfections. It is a liposome/polymer-based reagent with very low toxicity on transfected cells, making it an excellent choice of transfection reagent. In particular, this reagent is designed and manufactured for large working volume of transfection and large number of transfection events, including large scale protein production via transient transfection, high-throughput screening.

The major advantages of DNAFect LT include:

- Superior Efficiency: Ideal for transient or stable transfection in a variety of cell lines.
- Low Toxicity: The transfected cells remain healthy and produce more trangene protein.
- Simple Application: Suitable for serum-containing media; no requirement for media changes.
- High Affordability: Great cost-saving
- Effective on 293 and CHO suspension cells

Important Guidelines for Transfection

- We recommend DMEM or Opti-MEMI serum-reduced medium for serum-containing transfection events, and selected serum-free media (Invitrogen's Opti-pro SFM, CD OptiCHO™, FreeStyle™ CHO or 293) for no-serum transfection events to dilute DNAFect LT and DNA before complexing.
- We recommend not adding antibiotics to media during transfection.
- Maintain the same seeding density between experiments.

Transfection Protocol for Adherent Culture

Use the following procedure to transfect DNA into mammalian cells in a **24-well format**. For other formats, see Table 1. All amounts and volumes are given on a per well basis. Optimization may be required in some cases.

1. One day before transfection, plate 0.5-1.5 x 10^5 cells in 500 µl of growth medium so that cells will be 60-80% confluency at the time of transfection.
2. For each transfection event, prepare complexes as follows:

a. Dilute DNA in 50 µl of DMEM or Opti-MEMI medium without serum, or one of other recommended serum-free media.
b. Mix DNAFect LT gently before use, then dilutes the appropriate amount of DNAFect LT (see table below) in 50 µl of the same medium. Mix well and incubate for 5 minutes at room temperature.
c. After the 5 minute incubation, combine the diluted DNA with diluted DNAFect LT (total volume = 100 µl). Mix well manually or through vortexing and incubate for 10-15 minutes at room temperature.

3. Aspirate off old growth medium, wash once with 1 x PBS, and followed by adding 200 µl of one of selected growth media.
4. Add the 100 µl of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.
5. Incubate cells at 37°C in an incubator with 5% CO_2 overnight.
6. Add 700 µl of fresh growth medium into each well on the following day.
7. Incubate 36-72 hours prior to testing for transgene expression.
8. For stable cell lines: Passage cells at a 1:5 (or higher dilution) into fresh growth medium 24 hours after transfection. Add selective medium (if desired) on the following day.

To transfect cells in different cell culture formats, vary the amounts of DNAFect LT, DNA, cells, and medium used in proportion to the relative surface area, as shown in the Table 1.

Culture vessel	Surface area per well	Medium		Transfection	
		Planting volume	Dilution volume	DNA	DNAFect LT
96-well	0.3 cm^2	40 µl	2 x 10 µl	0.1 µg	0.3 µl
24-well	2 cm^2	200 µl	2 x 50 µl	0.6 µg	1.6 µl
12-well	4 cm^2	400 µl	2 x 100 µl	1.2 µg	3.2 µl
6-well/35-mm	10 cm^2	1.0 ml	2 x 250 µl	3.0 µg	8.0 µl
60-mm	20 cm^2	2.0 ml	2 x 0.5 ml	6.0 µg	16.0 µl
100-mm	60 cm^2	6.0 ml	2 x 1.5 ml	18.0 µg	48.0 µl

Table 1: Transfection component volumes for adherent culture.

Transfection Protocol for Suspension Culture

Follow the procedure below to transfect suspension cells in a 30 ml volume in a 125 ml shake flask; for other formats, see Table 2.

1. The day before transfection (day 1), determine the number of cells that you will need for your experiment. Remember that for each 30 ml transfection, you will need 3-4.5 x 10^7 cells in 30 ml in a selected serum-free growth medium (We recommend Invitrogen's Opti-pro SFM medium; Invitrogen's CD OptiCHO™, FreeStyle™ CHO or 293 medium can also be used). Expand the cells accordingly, taking into account the cell doubling time. Place the shaker flask containing cells in a 37°C incubator with 8% CO_2 on an orbital shaker.
2. On the day of transfection, transfer a small aliquot of the cell suspension to a microcentrifuge tube and determine cell density and viability using the trypan blue dye exclusion method. Dilute the cells to 1×10^6 /ml with growth medium. To ensure optimal transfection, viability of cells must be > 95%.

3. For each transfection sample, prepare DNAFect LT-DNA complexes by performing the following:
    a. Dilute 30 µg of plasmid DNA in Opti-pro SFM or Opti-MEM I medium to a total volume of 1 ml. Mix well.
    b. Dilute 60 µl of DNAFect LT in Opti-pro SFM or Opti-MEM I medium to a total volume of 1 ml. Mix gently and incubate for 5 minutes at room temperature.
    c. After the 5 minute incubation, add the diluted DNA to the diluted DNAFect LT to obtain a total volume of 2 ml. Mix well.
    d. Incubate for 10-15 minutes at room temperature to allow the DNA-DNAFect LT complexes to form.
4. Slowly add 2 ml of DNA-lipid mixture into the 125 ml flask containing cells while slowly swirling the flask.
5. Incubate transfected cell cultures at 37°C, 8% CO_2 on an orbital shaker set to 120-135 rpm overnight. There is no need to change or supplement the medium during the first 4 to 5 days. We recommend adjusting temperature to 29-33°C afterwards for cultivating cells until harvest.
6. Harvest cells and media (if recombinant protein is secreted) at certain days post-transfection and perform assays for transfection efficiency or recombinant protein expression. For GFP expression, we recommend performing assays 48~72 hr post-transfection. For secreted IgG production, we recommend harvesting media at 6~8 days post-transfection and perform assays.

To transfect cells in different cell culture formats, vary the amounts of DNAFect LT, DNA, cells, and medium used in proportion to the relative working volume, as shown in the Table 2.

Cell Culture		**Dilution**	**DNA**		**DNAFect LT**	
Volume	**Flask**	**Volume**	**Starting Point**	**Optimization Range**	**Starting Point**	**Optimization Range**
10 ml	50 ml	2x0.3 ml	10 µg	7-13 µg	20 µl	15-25 µl
30 ml	125 ml	2x1 ml	30 µg	20-40 µg	60 µl	45-75 µl
250 ml	1 liter	2x8 ml	250 µg	160-320 µg	500 µl	360-600 µl
1 liter	3 liter	2x32 ml	1 mg	0.6- 1.3 mg	2 ml	1.4-2.4 ml

Table 2: Transfection component volumes for suspension culture.

Shipping and Storage

DNAFect LT reagent is shipped at room temperature. Upon receipt, store this reagent at 4°C.