PerFectin™

Transfection Reagent

Genlantis

Catalog #	Contents	Quantity
T303007 (100 reactions)	PerFectin™ Lipid Film, dried	1 vial
	Hydration Buffer	1 x 0.8 ml
	DNA Diluent	1 x 5.5 ml
T303015 (200 reactions)	PerFectin™ Lipid Film, dried	1 vial
	Hydration Buffer	1 x 1.6 ml
	DNA Diluent	1 x 10.5 ml

Shipping	Shipped at room temperature.
Storage	Store at 4°C; stable for 1 year.

Related Products	Catalog #
GenePORTER® 2 Transfection Reagent, 75 reactions	T202007
GenePORTER® 2 Transfection Reagent, 150 reactions	T202015
GenePORTER® 2 Transfection Reagent, 750 reactions	T202075
BoosterExpress™ Reagents, 3 x 1.5 ml	T20100B
GeneSilencer® siRNA Transfection Reagent, 200 reactions	T500750
GeneSilencer® siRNA Transfection Reagent, 1000 reactions	T505750
phCMV1 Expression Vector Kit (native expression)	P003100
phCMV1 Expression Vector Kit (N-terminal fusion)	P003200
phCMV1 Expression Vector Kit (C-terminal fusion)	P003300
Enhanced β-galactosidase Assay Kit (CPRG)	A10100K
β -galactosidase Assay Kit (ONPG)	A10200K
X-gal Staining Assay Kit	A10300K
NeuroFect™ Transfection Reagent, 75-300 reactions	T800075
NeuroFect™ Transfection Reagent, 375-1500 reactions	T800750

Introduction: The PerFectin Transfection Reagent is a unique formulation of the neutral lipid, dioleoyl phosphatidylethanolamine (DOPE), and a proprietary cationic lipid derived from Genlantis' unique Direct Hydrophilic Conjugation (DHC) technology. The PerFectin Transfection Reagent has been specifically optimized for transfection and gene expression in CHO, COS, 293, NIH-3T3, HeLa, and Jurkat cells. Each kit includes a special DNA Diluent that increases transfection efficiency in the presence of serum. The PerFectin Transfection Reagent is suitable for both *in vitro* and *in vivo* transfection studies and offers the following benefits:

- **Superior transfection efficiency**
- **Compatibility with serum**
- **Cost effectiveness**
- **Extended shelf life**

METHODS AND PROCEDURES

I. PerFectin Lipid and DNA Solution Preparation

a. Hydrate PerFectin lipid film at room temperature with 0.75 ml of the hydration buffer. Vortex for 10 seconds at top speed before use. Store the hydrated reagent at 4°C and vortex briefly before each use.

b. Prepare the DNA solution by resuspending each 1 µg of DNA with 25 µl of the DNA Diluent.

c. Use 3.5 µl of PerFectin Reagent with 1 µg of DNA.

II. Example Protocols

For 293, CHO-K1, COS-7, HeLa-S3, and NIH-3T3 cells:

d. The day before transfection, plate the cells so that they will be ~60% confluent on the day of transfection.

e. Dilute the hydrated PerFectin reagent with serum-free medium according to Table 1 below:

Table 1: Amounts of DNA, Diluent, PerFectin, and Dilution Medium

DNA (µg)	DNA Diluent (µl)	PerFectin (µl)	Serum-free Medium (µl)
0.5	12.5	1.75	10.75
1.0	25.0	3.5	21.5
2.0	50.0	7.0	43.0
4.0	100.0	14.0	86.0
8.0	200.0	28.0	172.0

f. Dilute your DNA with the DNA Diluent according to Table 1 above. Mix well by pipetting several times, and incubate 5 minutes at room temperature.
NOTE: Avoid vortexing the DNA Diluent solution.

g. Add the DNA solution to the diluted PerFectin reagent. Incubate at room temperature for 5 minutes to form PerFectin/DNA complexes (lipoplexes).
NOTE: Do NOT incubate longer than 30 minutes.

h. Add the PerFectin/DNA complexes directly to the cells that are in serum-containing culture medium. Incubate at 37°C in a humidified atmosphere containing 5% CO_2.
NOTES: For some cells, such as HeLa-S3 for example, higher transfection efficiencies can be achieved when the initial 4-hour incubation is done in serum-free medium. For such cells, use the following four steps:

(i) Add serum-free medium to the complexes to make up the transfection volume (refer to Table 2 below).
(ii) Aspirate the culture medium from cells.
(iii) Add the complexes in serum-free medium to the cells. Incubate 4 hours at 37°C and 5% CO_2.
(iv) Add one volume of medium containing 20% serum. Then proceed to the next step.

Table 2: Transfection Volumes and DNA Amounts Per Dish Size

Tissue Culture Dish Size	DNA (μg)	Transfection Volume (ml)
96-well	0.1-0.5	0.1
24-well	0.5-2.0	0.25
6-well	2.0-6.0	1.0
60 mm	6.0-8.0	2.5
100 mm	8.0-12.0	5.0

i. 24 hours post transfection, add fresh growth media as needed. For some cell types, the old media can be replaced with fresh media at this step.

j. Assay for gene expression 24 to 72 hours following transfection.

NOTE: Depending on cell type and promoter activity, the same protocol can be used to produce stably transfected cells. To do so, put the cells in fresh medium containing the appropriate selection antibiotic 48 to 72 hours post transfection. It is important to wait at least 48 hours before exposing the transfected cells to the selection media.

For Jurkat Cells:

The protocol for Jurkat cells is similar to the protocols for other cells, with the following exceptions.

a. The day before transfection split the cells so they are in good condition on the day of transfection.

b. Prepare the mixture of PerFectin/DNA complexes as above. While the PerFectin/DNA complexes are incubating, spin down the cells, resuspend them at 1 x 10^6 or 2 x 10^6cells/ml in medium, and transfer the appropriate volume to the dish (Table 3). Serum-free medium is preferred to resuspend the cells.

Table 3: Number of Cells and Transfection Volumes for Jurkat

Tissue Culture Dish Size	Number of Cells Per Well	Transfection Volume (ml)
96-well	1 x 10^5	0.1
24-well	0.5 x 10^6	0.25
6-well	2 x 10^6	1.0
60 mm	5 x 10^6	2.5
100 mm	1 x 10^7	5.0

c. Add the lipoplexes directly to the cells, and mix well by gently pipetting 2 to 3 times. This step is important because Jurkat cells have a tendency to clump, and the reagent does not easily access cells in the center of clumps. Gentle pipetting of cells disrupts these clumps and produces a true single-cell suspension, which will increase transfection efficiency.

d. Incubate the cells at 37°C in a humidified atmosphere containing 5% CO_2.

e. Four hours post transfection; add one volume of medium containing 20% serum to the cells.

f. Add fresh growth media as needed 24 hours post transfection.

g. Assay the reporter gene 24 to 72 hours following transfection.

NOTE: If low expression levels are experienced, use serum-free medium instead of DNA Diluent to dilute the DNA. Also try lower DNA amounts, such as 1 μg of DNA per 7 μl of PerFectin reagent.

GENERAL NOTES

To obtain maximum efficiency in your particular cell lines, additional optimization may be needed. The two critical variables are the ratio of PerFectin reagent to DNA and the quantity of DNA. First maintain a fixed ratio of PerFectin reagent to DNA, and then vary the DNA quantity over the suggested range. If necessary, optimize the ratio of PerFectin reagent to DNA by using 2 to 6 μl of reagent for each 1 μg of DNA. Use a low DNA quantity to optimize this ratio. Following this process, cell number can also be optimized.