NEUROMICS

PROTOCOL FOR DNA DILUENTS

PF30000	p-Fect, DNA Diluent,	5x8 mls
PF30001	p-Fect, DNA Diluent,	10x8 mls
PF30002	p-Fect, DNA Diluent B,	5x8 mls
PF30003	p-Fect, DNA Diluent B,	10x8 mls, B

• Hydrate p-Fect lipid film at room temperature with hydration buffer. Vortex for 10 seconds at top speed before use. Store the hydrated reagent at 4oC and vortex briefly before each use.
• Use the DNA diluent to prepare the DNA solution. Use 25 µl of diluent for 1 µg DNA. Avoid vortexing the DNA diluent solution.
• For most cell types, use 5 µl of p-Fect reagent with 1 µg of DNA.

Transfection of adherent cells
1. Dilute the hydrated P-Fect reagent with serum-free medium.
• Refer to Table 2 for the appropriate volume of serum-free medium.
2. Dilute the DNA with the DNA diluent and incubate 1 to 5 minutes at room temperature. Do not
incubate longer than 5 minutes.
• Refer to Table 2 for the appropriate volume of DNA diluent.
• Avoid vortexing the DNA diluent.
3. Add the DNA solution to the diluted P-Fect reagent. Incubate at room temperature for 5 to 10 minutes to form p-Fect/DNA complexes (lipoplexes).
• Do not incubate longer than 30 minutes
4. Add the mixture of p-Fect/DNA complexes directly to the cells growing in serumcontaining culture medium.[a,b] Incubate at 37^{o}C.
• Refer to Table 3 for appropriate transfection volumes.
5. 24 hours post transfection, add fresh growth media as needed.c Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to72 hours following transfection.[d]

Table 2: Volumes of DNA Diluents[e]

DNA (µg)	DNA Diluent (µl)	p-Fect (µl)	Serum-free Medium (µl)
0.5	12.5	2.5	10.0
1.0	25.0	5.0	20.0
2.0	50.0	10.0	40.0
4.0	100.0	20.0	80.0
8.0	200.0	40.0	160.0

Table 3: Transfection Volumes and DNA Amount for Various Culture Dishes[e]

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

Notes:
[a]Cells plated the day before transfection should be 50% to 70% confluent on the day of transfection. Omitting antibiotics from the media during transfection may increase expression levels; this effect is cell-type dependent and usually small.
[b]For some cells (such as HeLa S3, MDCK, CHO-K1), higher transfection efficiencies can be achieved when the initial 4-hour incubation is done in serum-free media. After this step, add one volume of medium containing 20% serum, then proceed as in Step 5.
cFor some cell types, the old media can be replaced with fresh media at this step.
[d]The same protocol can be used to produce stably transfected cells: 48 to 72 hours post transfection, put the cells in fresh medium containing the appropriate selection antibiotic. It is important to wait at least 48 hours before exposing the transfected cells to the selection media. For some cell types it may be necessary to wait as long as 4 to 5 days before applying the selection condition.
[e]Although p-Fect reagent consistently delivers high transfection efficiencies in a wide range of cell types, in order to obtain maximum efficiency in particular cell lines, some optimization may be needed. The two critical variables are the ratio of p-Fect reagent to DNA and the quantity of DNA. For optimization, first maintain a fixed ratio of p-Fect reagent to DNA, then vary the DNA quantity over the suggested range. If necessary, optimize the ratio of p-Fect reagent to DNA by using 3 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.

Transfection of suspension cells
Although p-Fect reagent works well for cells such as K562 and PC 12, which can grow in suspension, it does not work well for Jurkat cells. For suspension cells, the protocol is the same as described for adherent cells, with the following exceptions:
1. The day before transfection, split the cells so they are in good condition on the day of transfection.
2. While the p-Fect/DNA complexes are incubating, spin down the cells, resuspend them at 1 x 10_6 or 2 x 10^6cells/ml in medium with or without serum, and transfer the appropriate volume to the dish (Table 4).
3. Prepare the mixture of p-Fect/DNA complexes as above, add it directly to the cells, and mix well by gently pipetting 2 to 3 times.[f] Incubate at 37°C and proceed as described for adherent cells.[g]

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

Table 4 Suggested Conditions for Transfecting Suspension Cells [h]

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

Notes:
[f]This step is important because some suspension 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.
[g]For some hematopoietic cell lines, mitogenic agents like PHA or PMA may be added to the cells 4 hours after transfection to a final concentration of 1 µg/ml or 50 ng/ml, respectively, to enhance the levels of gene expression.
[h]For suspension cells, the optimization procedure is the same as adherent cells (Table 3 and Notes[e]).

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com

Optional protocol for low quantity DNA transfection

The following revised protocol can be used to facilitate pipetting and transfer of DNA/lipids complexes to the cells when a low quantity of DNA (≤ 1 µg) is used for the transfection.

1. Dilute hydrated p-Fect reagent with serum-free medium (Table 5).
2. First dilute the DNA diluent in serum-free medium and then add the DNA. See Table 5 for volumes of serum-free medium, DNA diluent, and DNA amount. Incubate 1 to 5 minutes at room temperature.
3. Proceed as in Steps 3 though 5 as described for adherent cells.

Table 5 Recommended Amounts of Reagents for Optional Protocol

A: Dilution of p-Fect Reagent		
DNA (µg)	**Serum-free Medium (µl)**	**p-Fect Reagent (µl)**
0.125	49.37	0.63
0.25	48.75	1.25
0.5	47.5	2.5
1.0	45.0	5.0
B. DNA Dilution		
Serum-free Medium (µg)	**DNA Diluent (µl)**	**DNA (µg)**
46.8	3.12	0.125
43.75	6.25	0.25
37.5	12.5	0.5
25.0	25.0	1.0
C. Transfection Volume and DNA Amounts Per Dish Size		
Tissue Culture Dish Size	**DNA (µg)**	**Transfection Volume (ml)**
96 well	0.1-0.25	0.1
24 well	0.5-2.0	.25

Neuromics Antibodies • 5325 West 78th Street, Suite 8 • Edina, MN 55438
phone 952-374-6161 • fax 612-677-3976 • e-mail pshuster@neuromics1.com