

PROTOCOL FOR DNA DILUENT

DIRECTIONS

- Hydrate GenePORTER 2 lipid film at room temperature with hydration buffer. Vortex for 10 seconds at top speed before use. Store the hydrated reagent at 4°C 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 GenePORTER 2 reagent with 1 µg of DNA.

EXAMPLE PROTOCOLS

Transfection of adherent cells

1. Dilute the hydrated GenePORTER 2 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 GenePORTER 2 reagent. Incubate at room temperature for 5 to 10 minutes to form GenePORTER 2/DNA complexes (lipoplexes).
 - Do not incubate longer than 30 minutes
4. Add the mixture of GenePORTER 2/DNA complexes directly to the cells growing in serum-containing culture medium.^{a, b} Incubate at 37°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 to 72 hours following transfection.^d

Table 2

Volumes of Transfection Reagents^e			
DNA (µg)	DNA diluent (provided) (µl)	Gene-PORTER2 (µl)	Gene-PORTER2 diluent (Serum free medium) (µl)
0.5	12.5	2.5	10
1	25	5	20
2	50	10	40
4	100	20	80
8	200	40	160

Table 3

Transfection Volumes and DNA Amount for Various Culture Dishes^e		
Tissue Culture Dish	DNA (µg)	Transfection Volume (ml)
96-well	0.1-0.5	0.1
24-well	0.5-2	0.25
6-well	2-6	1
60 mm	6-8	2.5
100 mm	8-12	5

Notes:

^aCells 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.

^bFor 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.

^dThe 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.

^eAlthough GenePORTER 2 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 GenePORTER 2 reagent to DNA and the quantity of DNA. For optimization, first maintain a fixed ratio of GenePORTER 2 reagent to DNA, then vary the DNA quantity over the suggested range. If necessary, optimize the ratio of GenePORTER 2 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 GenePORTER 2 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 transfection of Jurkat cells, we recommend using the original GenePORTER reagent.

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 GenePORTER 2/DNA complexes are incubating, spin down the cells, resuspend them at 1×10^6 or 2×10^6 cells/ml in medium with or without serum, and transfer the appropriate volume to the dish (Table 4).
3. Prepare the mixture of GenePORTER 2/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

Table 4

Suggested Conditions for Transfecting Suspension Cells^h

Tissue Culture Dish	Number of Cells	Transfection Volume
96-well	1×10^5	0.1 ml
24 well	0.5×10^6	0.25 ml
6-well	2×10^6	1 ml
60 mm	5×10^6	2.5 ml
100 mm	1×10^7	5 ml

Notes:

^fThis 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.

^gFor 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.

^hFor suspension cells, the optimization procedure is the same as adherent cells (Table 3 and Notes^e).

Optional protocol for low quantity DNA transfection

The following revised protocol^{1, 2} 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 GenePORTER 2 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 through 5 as described for adherent cells.

Table 5
Recommended Amounts of Reagents for Optional Protocol

A. Dilution of GenePORTER 2 Reagent

DNA (µg)	Serum-free medium (µl)	GenePORTER 2 Reagent (µl)
0.125	49.37	0.63
0.25	48.75	1.25
0.5	47.5	2.5
1	45	5

B. DNA Dilution

Serum-free medium (µl)	DNA diluent (µl)	DNA (µg)
46.8	3.12	0.125
43.75	6.25	0.25
37.5	12.5	0.5
25	25	1

C. Transfection Volume and DNA Amounts for Different Plates

Tissue Culture Dish	DNA (µg)	Transfection Volume (ml)
96-well	0.1-0.25	0.1
24-well	0.5-2	0.25