GeneTrans II Transfection Reagent

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MOLECULAR BIOTECHNOLOGY

Contents

Introduction

The GeneTrans II Transfection Reagent is one of the most effective and widespread gene delivery reagents available. While offering all of the advantages of DHC technology as the original GeneTrans Reagent, the GeneTrans II Reagent delivers higher transfection efficiencies for more difficult to transfect cell types. With a choice of two optimized DNA diluent buffers, GeneTrans II performs effectively across a broad range of cells, offers the highest efficiencies, and is effective in the presence of serum, eliminating inconvenient media changes.

Protocols

DNA Diluent Selection

Select the DNA diluent suitable for your cell type as listed below; If no data is available for your cell type, try the DNA Diluent B protocol first.

Cell Lines	DNA Diluent	DNA Diluent B	Serum
HeLa-S3	★	★ ★	○
HeLa	★	★ ★	○
COS-1	★	★	●
COS-7	★	★	●
Hep-G2	★	★	●
NIH-3T3	★	★	●
MDCK	★	★ ★	○
K-562	★	★ ★	○
CV-1	★	★	●
B15-F0	★	★	●
293	★	★	●
BHK-21	★	★	●
CHO-K1	★ ▲	★ ▲	●
PC-12	★	NR	●
P19	★	★	●
HUVEC-C	★	★	●
Jurkat	♦	♦	○

Legend

★	Works well	○	Works well w/o serum
★★	Works better	●	Works well w/ and w/o serum
NR	Not recommended		
▲	Best expression is w/o serum during 1st hr of transfection		
♦	Original GeneTrans II reagent is recommended		

GeneTrans Lipid Hydration

1. Hydrate each GeneTrans II lipid vial at room temperature with 0.75 ml (for 0201B) or 1.5 ml (for 0202B and 0203B) of the Hydration Buffer. Vortex for 10 seconds at top speed before use. Store hydrated reagent at 4 °C; vortex briefly before each use.
2. Use 25 µl of the DNA Diluent or DNA Diluent B per each 1 µg of DNA. Avoid vortexing either of the DNA diluent solutions.

Protocol for using the DNA Diluent

3. For most cell types, use 5 µl GeneTrans II per 1 µg of DNA.
4. Your DNA can be suspended in TE buffer or purified water. A DNA concentration of at least 0.1 mg/ml works well for most reaction sizes.

Transfection of Adherent Cells

5. Dilute the hydrated GeneTrans II Reagent with serum-free medium as shown in Table 1 below.
6. Dilute the DNA with DNA Diluent as shown in Table 1 below; mix well by pipetting and incubate at room temperature for 1 - 5 minutes.

Note: *Do not vortex DNA Diluent.*

Table 1

Amounts of DNA, diluent, GeneTrans II, and Medium

DNA (µg)	DNA Diuent (µl)	GeneTrans II (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

7. Add the diluted DNA to the diluted GeneTrans II Reagent. Incubate at room temperature for 5 to 10 minutes to form lipid/DNA complexes (lipoplexes).

Note: *Do not incubate for more than 30 minutes*.

8. Add the GeneTrans II/DNA complexes directly to cells growing in serum-containing medium and incubate at 37 °C. Use Table 2 below for recommended transfection volumes and DNA amounts.

Note: *Cells plated the day before transfection should be 50% - 70% confluent on transfection day. Omitting antibiotics from the media during transfection may increase expression levels; this effect is cell-type dependent and usually small.*
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, followed by the addition of one volume of medium containing 20% serum.

Table 2

Transfection Volumes and DNA Amount per Dish Size

Tissue Culture Dish Size	DNA (µg)	Transfection Volume (ml)
96-well	0.1 - 0.5	0.10
24-well	0.5 - 2.0	0.25
6-well	2.0 - 6.0	1.00
60 mm	6.0 - 8.0	2.50
100 mm	8.0 - 12.0	5.00

9. Add fresh growth media as needed 24 hours post transfection; for some cell types, the old media can be replaced with fresh media at this step.

10. Depending on cell type and promoter activity, reporter gene assay can be performed 24 - 72 hours post transfection.

Note: *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. Wait at least 48 hours before exposing the transfected cells to the selection medium, and for some cell types it may be necessary to wait as long as 4 to 5 days.*

Transfection of Suspension Cells

GeneTrans II Reagent works well for most suspension cells, such as K562 and PC 12, but for Jurkat cells we recommend using the original GeneTrans Reagent.

For suspension cells, the protocol is the same as described for adherent cells, with the following exceptions:

11. The day before transfection, split the cells so they are in good condition on the day of transfection.
12. While the GeneTrans II/DNA complexes are incubating, spin down the cells, resuspend them at 1 x 10^6 or 2 x 10^6 cells/ml in medium with or without serum, and transfer the appropriate volume to the dish as indicate in Table 3 below.
13. Add the GeneTrans II/DNA complexes directly to the cells, and mix well by gently pipetting 2 to 3 times.

Note: *This step is important because some suspension cells have a tendency to clump, which reduces transfection efficiency.*

14. Incubate at 37 °C and proceed as described for adherent cells.

Note: *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.*

Table 3

Number of Cells and Transfection Volumes for Suspension Cells

Tissue Culture Dish	Number of Cells (µg)	Transfection Volume (ml)
96-well	1 x 10^5	0.10
24-well	0.5 x 10^6	0.25
6-well	2 x 10^6	1.00
60 mm	5 x 10^6	2.50
100 mm	1 x 10^7	5.00

Protocol for using the DNA Diluent B

15. For most cell types, use 3.5 µl of the GeneTrans II Reagent per 1 µg of DNA.

Transfection of Adherent Cells

16. Dilute the hydrated GeneTrans II Reagent with serum-free medium as shown in Table 4 below.
17. Dilute the DNA with DNA Diluent B as shown in Table 4 below; mix well by pipetting and incubate at room temperature for 5 minutes.

Note: *Do not vortex DNA Diluent B.*

Table 4

Amounts of DNA, diluent, GeneTrans II, and Medium

DNA (µg)	DNA Diluent B	GeneTrans II (µl)	Serum-free Medium (µl)
0.50	12.5	1.75	10.75
1.00	25.0	3.50	21.50
2.00	50.0	7.00	43.00
4.00	100.0	14.00	86.00
8.00	200.0	28.00	172.00

18. Add the diluted DNA to the diluted GeneTrans II. Incubate at room temperature for 5 minutes to form lipid/DNA complexes (lipoplexes).

Note: *Do not incubate for more than 30 minutes.*

19. Same as Step 8 above.
20. Same as Step 9 above.
21. Same as Step 10 above.

Transfection of Suspension Cells

Same as Transfection of Suspension Cells protocol on page 6 when using the DNA Diluent.

Detection of Expressed Reporter Genes

β-Galactosidase

The following protocol[1] is provided for your convenience.

Briefly, aspirate the culture media post transfection. Lyse the transfected cells from each well of a 96-well plate with 50 µl of the lysis buffer [0.1% Triton X-100 (w/v) in 250 mM Tris-HCl, pH 8.0], then subject the cells to one freeze-thaw cycle (freeze at -70 °C and thaw at room temperature). While the cells are being lysed, prepare a β-galactosidase (*E.coli*; Sigma) standard curve with 0.5% BSA in PBS (w/v). Once the plate of lysed cells is completely thawed, transfer a 50 µl aliquot of each point on the standard curve to control wells of the plate. Typically, β-galactosidase expression ranges from 10,000 to 2,000,000

pg. Develop color by adding 150 µl of 1 mg/ml chlorophenol red-β-D-galactopyranoside (CPRG; Boehringer Mannheim) dissolved in β-gal buffer (1 mM $MgCl_2$; 10 mM KCl; 50 mM β-mercaptoethanol; and 60 mM Na_2HPO_4, pH 8.0). Allow the reaction to proceed at room temperature until the red color develops (2 min to 4 hours, depending on cell type). Read absorbance at 580 nm.
An immunohistochemical approach for quantifying β-galactosidase has also been reported[3].

Green Fluorescent Protein

When green fluorescent protein (GFP) is the reporter gene used for transfections, use epifluorescence or confocal microscopy to detect expression. GFP has an excitation peak at 470 to 490 nm and emission peak at 510 nm. Expression levels of GFP can also be monitored by fluorescence-activated cell sorter analysis (FACS)[4].

Secreted Alkaline Phosphatase

When heat-stable secreted alkaline phosphatase (SEAP) is the reporter gene used for transfections, use the following assay: heat supernatants from transfected cells at 65 °C for 30 min to inactivate endogenous alkaline phosphatase activity. The SEAP transgene is stable during this treatment. Take aliquots of the culture media 48 hours post transfection, and determine the SEAP activity quantitatively by using a colorimetric assay based on hydrolysis of the chromogenic substrate para nitrophenyl phosphate (PNPP). Dissolve 1 mg/ml of PNPP reagent in a solution of 1 mM $MgCl_2$ and 100 mM diethanolamine, pH 9.8. Add 10 µl of 0.05% Zwittergent in PBS (free Ca^{2+} and Mg^{2+}) into each well of a 96-well plate. Then add 20 µl of the heated cell culture media to each well. For control wells, 20 µl of water is used to normalize the volume. An alkaline phosphatase standard (EIA grade calf intestine alkaline phosphatase; Boehringer Mannheim) can be used to generate a standard curve from 10 to 10,000 pg per well. Add 200 µl of the PNPP substrate to each well to start the enzymatic reaction. Allow the reaction to incubate at room temperature until the yellow color develops. Using 0.05% Zwittergent in PBS as the diluent virtually reduces the background to zero, which increases the detection limit of the assay. Read the plates at 405 nm using either kinetic or static mode.

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 transfection.

1. Dilute hydrated GeneTrans II Reagent with serum-free medium as in Table 5 below.
2. First dilute the DNA diluent in serum-free medium and then add the DNA. See Table 5 (A, B, and C) below for volumes of serum-free medium, DNA diluent, and DNA amounts.

Table 5: Recommended Amounts of Reagents for Optional Protocol

A. Dilution of GeneTrans II Reagent

DNA (µg)	Serum-free medium (µl)	GeneTrans II Reagent (µl)
0.125	49.37	0.63
0.250	48.75	1.25
0.500	47.50	2.00
1.000	45.00	5.00

B. DNA Dilution

Serum-free medium (µl)	DNA diluent (µl)	DNA (µg)
46.80	3.12	0.125
43.75	6.25	0.250
37.50	12.50	0.500
25.00	25.00	1.000

C. Transfection Volume and DNA Amounts per Dish Size

Tissue Culture Dish	DNA (µg)	Transfection Volume (ml)
96-well	0.1 - 0.25	0.10
24-well	0.5 - 2.00	0.25

3. Incubate 1 to 5 minutes at room temperature.
4. Proceed as in Steps 6 through 9 under the “Transfection of Adherent Cells” Section (when using the DNA Diluent).

References

Cheng, L., *et al*. Use of green fluorescent protein variants to monitor gene transfer and expression in mammalian cells. *Nature Biotechnology*. 1996; 14:606-609.

Felgner, PL, *et al*. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci USA* 1987;84:7413-7417.

Felgner, JH, *et al*. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. *J Biol Chem*. 1994; 269:2550- 2561.

Gussoni, E., *et al*. A method to codetect introduced genes and their products in gene therapy protocols. *Nature Biotechnology*. 1996; 14:1012-1015.

Available Kits and Contents

GeneTrans II Transfection Reagent (75 reactions)		
Kit contents	GeneTrans II Lipid Film, dried	1 vial
	Hydration Buffer, Transfection Grade	1 x 0.8 ml
	DNA Diluent	1 x 4.0 ml
	DNA Diluent B	1 x 4.0 ml

GeneTrans II Transfection Reagent (150 reactions)		
Kit contents	GeneTrans II Lipid Film, dried	1 vial
	Hydration Buffer, Transfection Grade	1 x 1.6 ml
	DNA Diluent	1 x 8.0 ml
	DNA Diluent B	1 x 8.0 ml

GeneTrans II Transfection Reagent (750 reactions)		
Kit contents	GeneTrans II Lipid Film, dried	5 vials
	Hydration Buffer, Transfection Grade	5 x 1.6 ml
	DNA Diluent	5 x 8.0 ml
	DNA Diluent B	5 x 8.0 ml

Order Information, Shipping, and Storage

Order#	Product	Quantity
0201B	GeneTrans II Transfection Reagent	75 rxns
0202B	GeneTrans II Transfection Reagent	150 rxns
0203B	GeneTrans II Transfection Reagent	750 rxns
shipped at RT; store at 4 °C		

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