GeneTrans II QuickEase Kit

Order #
0204B

Mo Bi Tec
MOLECULAR BIOTECHNOLOGY

Contents

Description

GeneTrans II QuickEase Kit is a unique formulation of the neutral lipid dioleoyl phosphatidylethanolamine (DOPE) and a proprietary cationic lipid derived from the patented direct hydrophilic conjugation (DHC) technology. The GeneTrans II QuickEase Kit contains 96 single reaction tubes, which makes transfection easier and more convenient. Each tube contains sufficient GeneTrans II QuickEase Kit for transfecting 6 µg or less of DNA.

Components

Dried GeneTrans II lipid film (96 tubes)
DNA diluent (6 ml)
DNA diluent B (8 ml)

Storage: Store components at 4 °C.

Stability: Dried GeneTrans II QuickEase Kit is stable for at least 1 year at 4 °C.
DNA diluent and DNA diluent B are stable for at least 6 months at 4 °C.

Introduction

GeneTrans II QuickEase Kit is the newest advance in gene delivery developed by MoBiTec.
While featuring all of the advantages of DHC technology as the original GeneTrans QuickEase Kit, GeneTrans II QuickEase Kit delivers higher expression levels than other commercially available products, especially in difficult-to-transfect cell lines. With the two optimized DNA diluents, GeneTrans II QuickEase Kit performs over a broad range of cell types with or without the presence of serum.

- Highest efficiencies in diverse cell types
- Best with difficult-to-transfect cell lines
- Excellent performance in presence of serum
- Convenient and easy protocols
- Extended shelf life

Examples of Transfected Cell Types

Transfected Cell Types	
HeLa S3	BHK-21
293	CHO-K1
MDCK	CV1
NIH 3T3	COS-1
B16-F0	COS-7
PC-12	HepG2
K562	P19
HeLa	HUVEC-C

GeneTrans II QuickEase Kit was successfully used to transfect β-galactosidase reporter gene into many cell lines.

Directions

- Use the DNA diluent to prepare the DNA solution. Choose appropriate diluent according to the following table. If your cell type is not listed, start with the DNA Diluent B.
- Hydrate GeneTrans II dry film in the tube with the DNA solution.
- Add more medium to the DNA/GeneTrans II complexes, transfer the complexes onto the cells.

Recommended uses for DNA Diluents

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 QuickEase Kit is recommended		

Protocols

Transfection of adherent cells (6-well plates)

1. Plate cells so that they will be 50 - 70% confluent on the day of transfection.
2. Dilute the 4 µg DNA with 100 µl DNA Diluent or 6 µg DNA with 150 µl DNA Diluent B. Incubate 5 minutes at room temperature.
3. Add serum-free medium to the diluted DNA to bring up the volume to 250µl.
4. Hydrate the dry GeneTrans II QuickEase Kit with the DNA solution, pipette up and down 5 times. Incubate at room temperature for 10 - 20 minutes to form GeneTrans II/DNA complexes (lipoplexes).
5. Add the GeneTrans II/DNA complexes directly to the cells growing in serum-containing culture medium. The following table indicates the suggested volumes to use per well for various tissue culture plates. If using a tissue culture plate with wells smaller than those for 6-well plates, subdivide the lipoplex volumes as indicated. Add the appropriate amount of medium to each well to bring the volume up to the total transfection volume indicated.

Table 1 Suggested Transfection Volumes and DNA Amounts

Tissue culture dish	Lipoplex volume/well	DNA amount/well	Total transfection volume/well
6-well	250 µl	4 µg	1 ml
12-well	125 µl	2 µg	500 µl
24-well	62.5 µl	1 µg	250 µl
96-well	31.25 µl	0.5 µg	100 µl

6. Incubate at 37 °C.
7. 24 hours post transfection, add fresh growth media as needed [b]. Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to 72 hours following transfection[c].

Notes:

a 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.

b For some cell types, the old media can be replaced with fresh media at this step.

c 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 antibiotics. 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.

Suggested DNA optimization ranges for different tissue culture plates:

- 6-well. Transfer 3 - 5 µg of DNA directly into each well and adjust the final volume to 1 ml.
- 12-well. Transfer 1 - 3 µg of DNA directly into each well and adjust the final volume to 500µl.
- 24-well. Transfer 0.5 - 1.5 µg of DNA directly into each well and adjust the final volume to 250µl.

- 96-well. Transfer 0.1 - 0.5 µg of DNA directly into each well and adjust the final volume to 100µl.

Transfection of suspension cells (6-well) GeneTrans II QuickEase Kit works well for cells such as K562 and PC 12, which can grow in suspension. For Jurkat cells, we recommend using the original GeneTrans II QuickEase Kit. 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 GeneTrans II /DNA complexes are incubating, spin down the cells, resuspend cell numbers indicated in Table 2 below in medium with or without serum, and transfer the complexes to the dish.
3. Prepare the GeneTrans II/DNA complexes as above, add the complexes directly to the cells, and mix well by gently pipetting 2 to 3 times.d Incubate at 37 °C and proceed as described for adherent cells [e].

Table 2 Suggested Cell Numbers, Transfection Volumes and DNA Amounts

Tissue Culture Dish Size	Cell number/ well	Lipoplex volume/well	DNA amount/well	Total transfection volume/well
6-well	2,000,000 cells	250 µl	4 µg	1 ml
12-well	1,000,000 cells	125 µl	2 µg	500 µl
24-well	500,000 cells	62.5 µl	1 µg	250 µl
96-well	100,000 cells	31.25 µl	0.5 µg	100 µl

Notes:

d This step is important because some suspension cells have a tendency to clump, and the reagent has difficulty getting access to the cells in the center of these clumps. Gentle pipetting of cells disrupts these clumps and produces a true single-cell suspension, which will increase transfection efficiency.

e 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.

Suggested DNA optimization ranges for different tissue culture plates:

- 6-well. Transfer 3 - 5 µg of DNA directly into each well and adjust the final volume to 1ml.
- 12-well. Transfer 1 - 3 µg of DNA directly into each well and adjust the final volume to 500 µl.
- 24-well. Transfer 0.5 - 1.5 µg of DNA directly into each well and adjust the final volume to 250 µl.
- 96-well. Transfer 0.1 - 0.5 µg of DNA directly into each well and adjust the final volume to 100 µl.

Order Information, Shipping and Storage

Order#	Product	Quantity
0204B	GeneTrans II QuickEase Kit	96 rxns
shipped at RT; store at 4 °C		

Related Products

Reagent for enhanced transfection efficiency
GenePusher Reagent Kit is a set of chemical cocktails designed to significantly increase transfection efficiencies with any non-viral DNA transfection reagent. Simply add the appropriate GenePusher reagent to the culture medium 4 hours post transfection and get up to 12 times enhancement of gene expression levels. Each kit includes three GenePusher reagents and a comprehensive manual.

Product	**Catalog #**
GenePusher Reagent Kit	CB-TDT10666-01

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