NTERA-2 CL.D1 Cell Avalanche® Transfection Reagent

Cat. No. EZT-NTER-1 **Size: 0.5 ml** **Store at 4°C**
1.5 ml

Cell Line Information:

Designations: NTERA-2 cl.D1 [NT2/D1]
Depositors: PW Andrews
Growth Properties: adherent
Organism: Homo sapiens
Morphology: epithelial-like, differentiation changes phenotype
Source: Organ: testis
Disease: malignant pluripotent embryonal carcinoma
Derived from metastatic site: lung
Applications: transfection host
Virus Resistance: UNTREATED CELLS: human cytomegalovirus (HCMV); human immunodeficiency virus (HIV-1, HTLV-III)
Age: 22 years
Gender: male
Ethnicity: Caucasian
Comments: The NTERA-2 cl.D1 cell line is a pluripotent human testicular embryonal carcinoma cell line derived by cloning the NTERA-2 cell line. The parental NTERA-2 lines was established in 1980 from a nude mouse xenograft of the Tera-2 cell line. This clone differentiates along neuroectodermal lineages after exposure to retinoic acid (RA) or hexamethylene bisacetamide ((HMBA). The RA induced differentiation is characterized by glycolipid changes, appearance of neurons, and induction of homeobox (HOX) gene clusters. The cells exhibit high expression of N-myc oncogene activity.

The Transfection Reagent:

Transfection of pluripotent stem cells or stem-like cells, such as NTERA-2 cl.D1 cell line, has always been challenging, both technically and financially. The most common systems currently used for molecular delivery to these cells include expensive electroporation instruments and relatively toxic chemical formulations.

NTERA-2 cl.D1 Cell Avalanche™ Transfection Reagent is a new, proprietary solution specifically designed for transfection on NTERA-2 cl.D1 cells. The proprietary formulation of lipids and polymers ensures the highest possible transfection efficiencies and viabilities for NTERA-2 cl.D1 cells.

Features:

- Specifically optimized to deliver nucleic acids into NTERA-2 cl.D1 cells
- Highest efficiency to ensure experimental success
- Lowest Cellular Toxicity-maintain cell density and reduce experimental biases
- 0.5 ml is able to transfect about 1000 wells of 24-well plate
- Deliver single or multiple plasmids
- Synthesized from 100% animal origin-free components, making it easy to validate the absence of zoonotic diseases, such as BSE or viruses, in research experiments or cells lines
- Economical: High efficiency means less amount of nucleic acid & reagent is needed
- Developed and manufactured by EZ Biosystems

BEFORE YOU START:

Important Tips for Optimal Transfection

1. Prepare high-quality plasmid DNA at 0.5–5 µg/µl in deionized water or TE buffer. A GFP (green fluorescent protein) plasmid can be used to determine transfection efficiency.
2. Use Opti-MEM® I Reduced Serum Medium (Life Technologies) or regular DMEM without serum to make The Reagent and nucleic acid mix (Only Opti-MEM® I will be mentioned in the remaining parts of the protocol for simplification purpose). Do not use $NaCl_2$ solution or PBS.
3. Maintain the same seeding conditions between experiments. Use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection.
4. The Reagent is extremely gentle to cells. However, transfection process will impose stress on cells, no matter what type of transfection methods you use. The trick is to get the balance between transfection efficiency and cell viability. Increasing the number of cells plated per well or decreasing DNA/The Reagent amount will minimize the effect of transfection on cell growth and viability. With careful adjustment, as described in page 3 and 4, this can be achieved while keeping the highest transfection efficiency.
5. Don’t use antibiotics in the culture medium during the first 24 hours of transfection.

Protocols

1 DNA Transfection

1.1 Cell Seeding

For optimal DNA transfection conditions, we recommend using cells which are 70% to 90% confluent at the time of transfection. Typically, for experiments in 24-well plates, $1.0x10^5$-$1.6x10^5$ cells are seeded per well in 0.5 ml of cell growth medium **without antibiotics** 24 h prior to transfection. For other culture formats, refer to Table 1.

Table 1. Recommended number of cells to seed the day before transfection

Culture vessel	Number of cells to seed	Surface area per well (cm^2)	Volume of medium per well to seed the cells (ml)
96-well	15,000-20,000	0.3	0.1
24-well	$1.0x10^5$-$1.6x10^5$	1.9	0.5
12-well	$1.6x10^5$-$3x10^5$	3.8	1
6-well/35 mm	$3x10^5$-$5x10^5$	9.4	2
60 mm/flask 25 cm^2	$5x10^5$-$1.6x10^6$	25-28	5
100 mm/flask 75 cm^2	$2x10^6$-$4x10^6$	75-78.5	10
150 mm/flask 175 cm^2	$4x10^6$-$1.0x10^7$	153-175	25

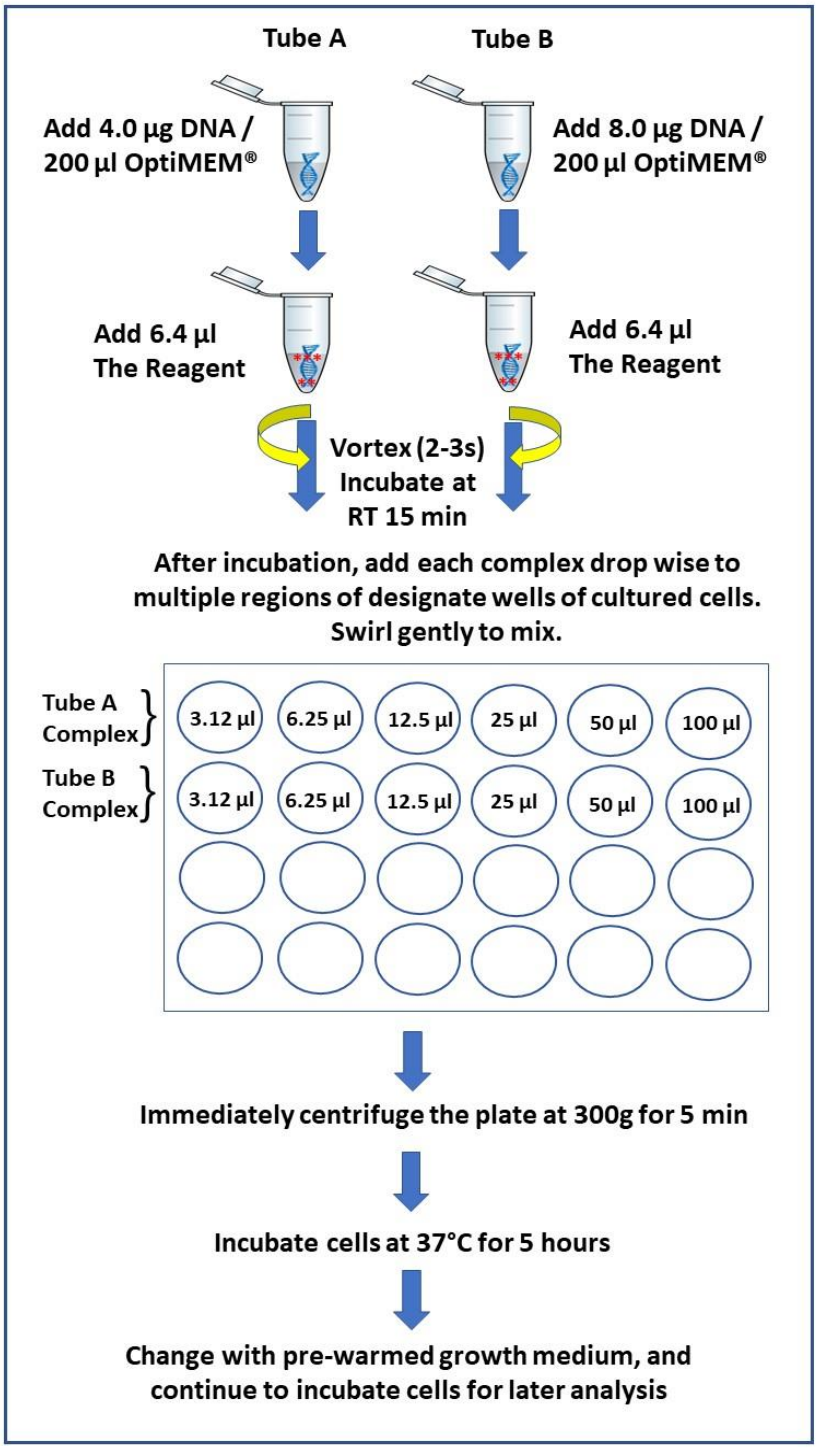
1.2 DNA Transfection on NTERA-2 CL.D1 cells

Due to cell culture variations and passage number differences, NTERA-2 CL.D1 cells from different sources may have different sensitivity to The Reagent. If this is the first time that you are using The Reagent on your NTERA-2 CL.D1 cells, follow the following procedures and Figure 1 for fine-tuning optimization (**The fine-tuning optimization procedures are extremely important for successful transfection. The amount of DNA/The Reagent needed for maximum transfection on NTERA-2 CL.D1 cells from different sources may differ dramatically**).

As an example, the following procedures and Figure 1 are for fine-tuning optimization on 24-well plate:

1. Bring The Reagent and serum-free medium (OptiMEM®I) to room temperature before starting.
2. Add 200µl of OptiMEM®I into two 1.5ml tubes (Tube A and B).
3. Add 4.0 µg of DNA to Tube A and 8.0 µg of DNA to Tube B.
4. Mix The Reagent prior to preparing complexes. Add 6.4 µl of The Reagent to both Tube A and Tube B containing 200µl of different concentration of DNA solution (20.0 µg/ml and 40.0 µg/ml respectively). Vortex for 2-3 seconds. Incubate each tube for 15 minutes at room temperature.
5. After incubation, add 3.12, 6.25, 12.5, 25, 50, and 100µl of DNA/The Reagent complexes dropwise directly to the corresponding wells of the 24-well cell culture plate (See Figure 1). Swirl plate gently.
6. Immediately centrifuge the plate at 300 g for 5 min.
7. Incubate the cells at 37°C in a CO2 incubator 5 hours.
8. Change growth medium, and continue to culture in the incubator.
9. Expression of reporter gene activity should generally be assessed at 20-48 hours post-transfection. GFP expression is maximal at 40-48 hours post-transfection.

Figure 1.



After you have completed the fine-tuning optimization steps, choose the amount of DNA and The Reagent that gave you the optimal balance of potency & low cytotoxicity (which usually is the lowest dose that gave you the same high transfection efficiency as other higher doses did) for all of your future experiments on this specific cell type.

1.3 Scale Up or Down Transfections

Use Table 2 to scale the amount of DNA/The Reagent for your transfection experiment.

Table 2. Scaling Up or Down Transfection Instruction

Culture Vessel	Multiplication factor*
96-well	0.17
48-well	0.50
24-well	**1.00**
12-well	2.00
6-well	5.00
60-mm	11.05**
10-cm	28.95**
T75	39.47**

*****After determining the optimum amount of DNA/The Reagent from the fine-tuning optimization procedures on the above 24-well plate, use the multiplication factor to determine the DNA and The Reagent amount needed for your new plate format.*

******For large format transfections, the centrifugation step may be skipped without obviously affecting transfection efficiency.*

1.4 DNA Transfection on cells other than NTERA-2 CL.D1 cells

NTERA-2 CL.D1 Cell Avalanche® Transfection Reagent can also be used on the following cells with high transfection efficiencies.

786-O Cell, Caki-1 Cell, MDCK Cell, Vero Cell, DU 145 Cell, 22Rv1 Cell, VCaP Cell, PC-3 Cell, AR42J Cell

The above protocol usually provides satisfactory transfection efficiency with invisible cytotoxicity on the above cells. However, additional optimization may be needed for certain type of cells. Optimizations may include: cell density; the amount of DNA and The Reagent; DNA/The Reagent ratio, or incubation time for the mixture of The Reagent/DNA etc.

If you want to achieve the best transfection result for a specific type of cells, we recommend using the respective cell type/cell line specific Avalanche® transfection reagents. Those reagents have been optimized on both recipes and protocols, and have been proved to have the best transfection results for the respective cell lines or primary cells. You can easily find the respective Avalanche® Transfection Reagents specific for your cells by using the filters of our product list in EZ Biosystems website: www.ezbiosystems.com.

2 siRNA Transfection

Though NTERA-2 CL.D1 Cell Avalanche® Transfection Reagent formulation was optimized for DNA transfection, it can also be used for siRNA transfection. The following are recommended protocol. Protocol optimization may be needed.

2.1 Cell Seeding

For optimal siRNA transfection conditions, we recommend using cells which are 50% confluent at the time of transfection. Typically, for experiments in 6-well plates, 100 000 to 150 000 cells are seeded per well in 2 ml of growth medium without antibiotics 24 h prior to transfection. For other culture formats, refer to Table 3.

Table 3. Recommended number of cells to seed the day before transfection.

Culture vessel	Number of cells to seed	Surface area per well (cm^2)	Medium per well to seed the cells (ml)
24-well	$5x10^4$-$8x10^4$	1.9	0.5
12-well	$1.0x10^5$-$1.6x10^5$	3.8	1
6-well/35 mm	$2.0x10^5$-$3.0x10^5$	9.4	2
60 mm/flask 25 cm^2	$4.0x10^5$-$1.0x10^6$	25-28	5
100 mm/flask 75 cm^2	$1.0x10^6$-$2x10^6$	75-78.5	10

2.2 siRNA Transfection

We recommend using 10 to 50 nM siRNA (final concentration). The following conditions are given per well of a 6-well plate. For other culture format, please refer to Table 4.

1. Change fresh media before transfection
2. Dilute 22 to 110 pmoles siRNA (final concentration: 10 to 50 nM) into 200 µl of Opti-MEM® Reduced-Serum Medium or regular high glucose DMEM without serum. Mix by vortexing.
3. Briefly vortex The Reagent, and add 1.0-5.0 µl into the diluted siRNA. Immediately vortex for 10 s.
4. Incubate for 15 min at RT.
5. Add the transfection mixture drop-wise into each well.
6. Gently rock the plates back and forth and from side to side, and immediately centrifuge the plate at 300 g for 5 min.
7. Gently put in incubator, and incubate at 37 °C CO2 for 5 hours.
8. Remove the media containing the transfection mixture, and add pre-warmed normal serum-containing fresh media. Continue to culture for 24 hours or more for analysis.

Table 4. siRNA transfection guidelines according to the cell culture vessel per well

Culture Vessel	siRNA (pmole) 10 nM	siRNA (pmole) 50 nM	The Reagent (µl)	Opti-MEM or DMEM (µl))	Growth medium (ml)	Final Volume in the well (ml)
24-well	5.5	27.5	*0.2-1.0	50	0.5	0.55
12-well	11	55	*0.4-2.0	100	1	1.1
6-well/ 35 mm	22	110	1.0-5.0	200	2	2.2
60 mm/ flask 25 cm^2	44	220	2.3-11.5	400	4	4.4
100 mm/ flask 75 cm^2	121	605	5.8-29	1100	11	12.1

* Dilute The Reagent 1:5 with H_2O prior application (4 µl reagent + 16 µl H_2O), and then use 5 times of volume for accurate pipetting.