PC-12 Cell Avalanche® Transfection Reagent

Cat. No. EZT-PC12-1 **Size: 0.5 ml** **Store at 4°C**
1.5 ml

Cell Line Information:

Designations: PC-12
Organism: Rattus norvegicus (rat)
Tissue: pheochromocytoma; adrenal gland
Gender: male
Morphology: small irregularly shaped cells
Tumorigenic: yes, in New England Deaconess Hospital strain rats
Karyotype: 40 chromosomes; 38 autosomes plus XY
Products: catecholamines; dopamine; norepinephrine
Receptors expressed: nerve growth factor (NGF)
Growth Properties: floating clusters; few scattered lightly attached cells.
Depositor: B. Patterson
Comments: The PC-12 cell line was derived from a transplantable rat pheochromocytoma. The cells respond reversibly to NGF by induction of the neuronal phenotype when plated on Collagen IV coated culture flasks. The cells do not synthesize epinephrine.

The Transfection Reagent:

Transfection of suspension cells or suspension/adherent mixed cells, such as PC-12 Cells, has always been challenging, both technically and financially. The most common systems currently used for relatively efficient molecular delivery to these cells are expensive electroporation instruments. However, electroporation usually leads to cell death, primarily when the electrical fields cause permanent permeabilization of the membrane and the consequent loss of cell homeostasis, in a process known as irreversible electroporation.

PC-12 Cell Avalanche® Transfection Reagent (Hereafter “The Reagent”) is a new class of unique chemical formulations specifically formulated and optimized for transfecting PC-12 cells. The proprietary formulation of lipids and polymers ensures the highest possible transfection efficiencies and viabilities for PC-12 cells.

Features:

- Specifically optimized to deliver nucleic acids into PC-12 cells
- Highest efficiency to ensure experimental success
- Extremely gentle to cells
- Deliver single or multiple plasmids
- 100% animal-free
- Compatible with serum
- Suitable for Reverse Transfection
- Compatible with transfection in any plate formats
- Reproducible: due to highly controlled chemical synthesis of each of the ingredients, the reagent forms uniformly sized complex particles with nucleic acids. With optimized protocol, our reagent will ensure the reproducible highest transfection results.
- 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, use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection. Typically, for experiments in 24-well plates, about $2x10^5$ cells are seeded per well in 0.5 ml of fresh medium **without antibiotics** 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	*4 x 10^4 **(7,500-10,000)	0.3	0.1
24-well	*2 x 10^5 **(50,000-80,000)	1.9	0.5
12-well	*4 x 10^5 **(80,000-150,000)	3.8	1
6-well/35 mm	*8 x 10^5 **(150,000-250,000)	9.4	2
60 mm/flask 25 cm^2	*2 x 10^6 **(250,000-800,000)	25-28	5
100 mm/flask 75 cm^2	*6 x 10^6 **($1x10^6$-$2x10^6$)	75-78.5	10
150 mm/flask 175 cm^2	*1.3 x 10^7 **($2x10^6$-$5x10^6$)	153-175	25

*For PC-12 cells

**For adherent culture cells (see section 1.4)

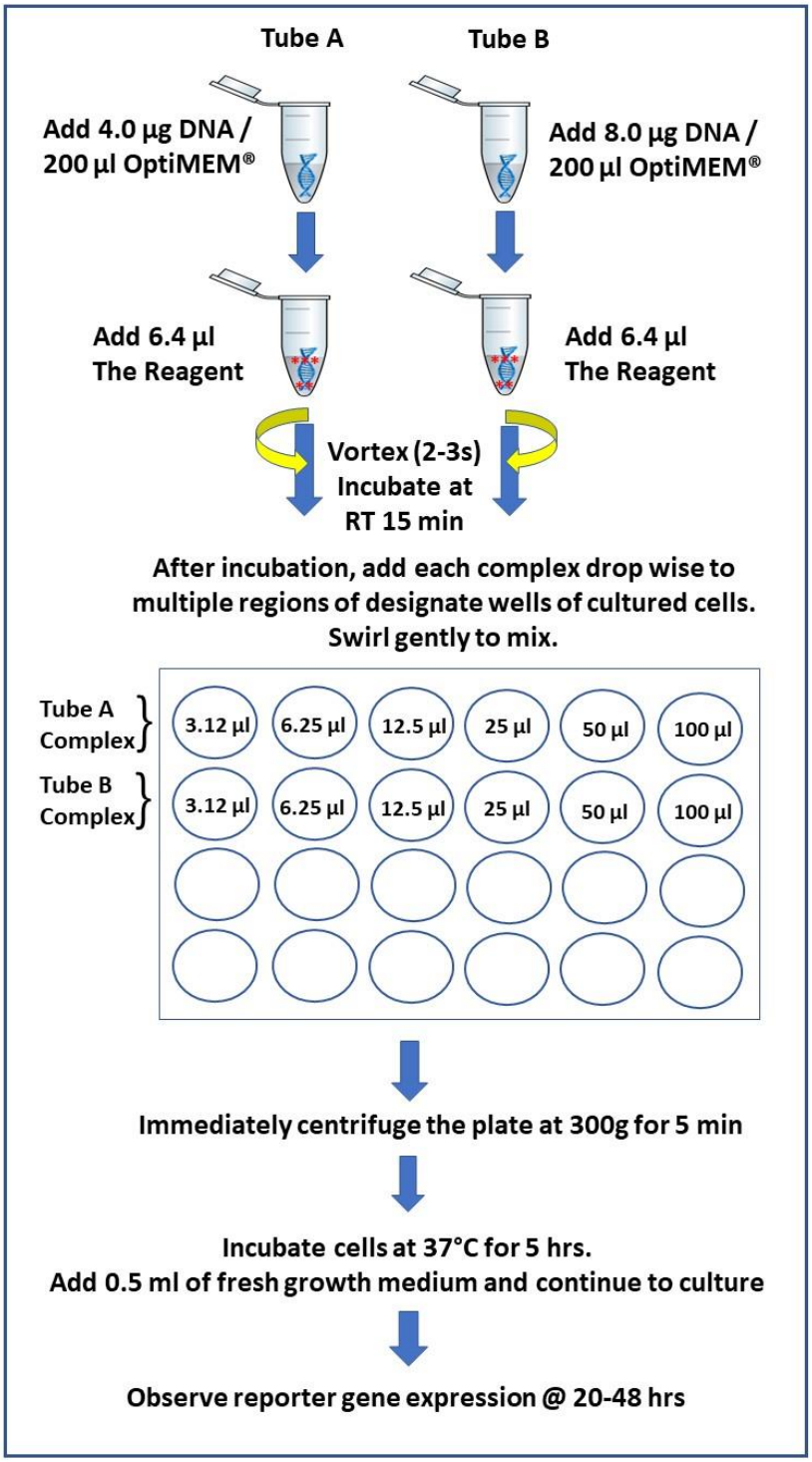
1.2 DNA Transfection on PC-12 cells

Due to cell culture variations and passage number differences, PC-12 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 PC-12 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 PC-12 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. Add 0.5 ml of fresh growth medium, and continue to culture.

Figure 1.



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.

After you have completed the fine-tuning optimization steps, choose the amount of DNA/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 PC-12 cells

PC-12 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, 293 Cell, 293T/17 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 formulations 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 PC-12 Cell Avalanche® Transfection Reagent formulation was optimized only 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, use low-passage cells; make sure that cells are healthy and greater than 90% viable before transfection. Typically, for experiments in 6-well plates, about 5 x 10^5 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	1.25 x 10^5	1.9	0.5
12-well	2.5 x 10^5	3.8	1
6-well/35 mm	5 x 10^5	9.4	2
60 mm/flask 25 cm^2	1.25 x 10^6	25-28	5
100 mm/flask 75 cm^2	3.75 x 10^6	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. 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.
2. Briefly vortex The Reagent, and add 1.0-5.0 µl into the diluted siRNA. Immediately vortex for 10 s.
3. Incubate for 15 min at RT.
4. Add the transfection mixture drop-wise into each well.
5. Gently rock the plates back and forth and from side to side, and immediately centrifuge the plate at 300 g for 5 min.
6. Gently put in incubator, and incubate at 37 °C CO_2.
7. Analyze after 24 h or later.

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.