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PeneFect™ Transfection Reagent

Cat. #: M0001 Size: 1 ml/5 ml

Protocol for generation of rAAV from 293T cell

Introduction:

Based on our innovative polymer synthesis technology, PeneFect™ Plus In Vitro DNA Tranfection Reagent is formulated to be a powerful transfection Reagent that ensures effective and reproducible transfection with less cytotoxicity. PeneFect™ reagent was shown to deliver DNA to various established cell lines as well as primary cells. PeneFect™ reagent was shown to generate rAAV with extremely high titers from 293T cells.

Important Guidelines for Transfection:

- For high titer of rAAV, 293T cell must be healthy. Please grow the 293T cell per supplier's instruction.

- For high efficiency, transfect cells at high density. ~80% confluency is highly recommended.

- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics.

- Use serum-free DMEM with High Glucose to dilute PeneFect™ reagent and DNA. The diluent must be serum-free.

Procedures for Transfecting 293T Cells

Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~80% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection. (see **Table 1**)

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. For some specific 293 cells, maximal transfection efficiencies are observed in the presence of serum and antibiotics. We recommend using complete serum/antibiotics-containing medium initially.

Preparation of PeneFect™/DNA Complex and Transfection Procedures:

The following protocol is given for transfection in 150 mm dish. For other culture formats, scale up or down per culture dish's surface. The optimal transfection conditions are given in the standard protocol described below.

- Cell confluency should be ~80 % at the day of transfection.

- For each 15 cm dish, add 15 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.

Table 1. A Guideline for Seeding Adherent Cells Prior to Transfection in Different Culture Formats.

Culture Dish	Surface Area (cm^2)	Number of Cells to Seed
T75 Flask	75	$3.0 - 6.0 \times 10^6$
100 mm dish	58	$2.2 - 4.4 \times 10^6$
60 mm dish	21	$0.9 - 1.8 \times 10^6$
35 mm dish	9.6	$3.5 - 7.0 \times 10^5$
6-well plate	9.6	$4.0 - 8.0 \times 10^5$
12-well plate	3.5	$1.5 - 3.0 \times 10^5$
24-well plate	1.9	$0.8 - 1.6 \times 10^5$
48-well plate	1.0	$4.0 - 8.0 \times 10^4$

- For each dish, dilute 10 µg of rAAV cis plsamid, 10 µg capsid DNA and 16 µg helper DNA (total 36 µg DNA) in 750 µl serum-free DMEM with high glucose. Vortex gently to mix.

- For each dish, dilute 100 µl of PeneFect™ reagent into 750 µl of serum-free DMEM with high glucose. Vortex gently to mix.

Note: Never use Opti-MEM to dilute DNA and PeneFect™ reagent because it will disrupt transfection complex.

- Add the diluted PeneFect™ Reagent immediately to the diluted DNA solution all at once. (Important: do not mix the solutions in the reverse order)

- Immediately pipette up and down 3~4 times or vortex briefly to mix followed by incubation for ~10 minutes at room temperature to allow DNA/PeneFect™ transfection complex to form.

Note: Never keep the DNA/PeneFect™ complex longer than 20 minutes

- Add the 1500 µl PeneFect™/DNA complex drop-wise onto the medium in each dish and homogenize the mixture by gently swirling the plate.

- Remove DNA/PeneFect™ complex-containing medium and replace with fresh complete serum/antibiotics containing medium 5 hours post transfection.

- Check transfection efficiency and virus titer 24 to 48 hours post transfection. 48 hours gives better titers.

Storage: PeneFect™ Transfection Reagent is stable for up to 12 months at 4°C.

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