

Introduction:

Based on our innovative polymer synthesis technology, PeneFect™ Transfection Reagent is formulated to be a powerful transfection reagent that ensures effective and reproducible transfection with less cytotoxicity. PeneFect™ was shown to deliver genes to various established cell lines as well as primary cells.

Important Guidelines for Transfection:

- PeneFect™ reagent was formulated for DNA transfection ONLY! The following standard protocol is for transfecting mammalian cells. To request protocol for lentivirus, rAAV or adenovirus production, please email us at info@lifescb.com
- For better efficiency, choosing a correct protocol is essential. We strongly encourage to use “General Protocol” first. If the “General Protocol” fails to give satisfactory result (e.g., less than 10%), try the “Advanced Protocol” in the back page
- For high efficiency and lower toxicity, transfect cells at high density. 70~80% confluency is highly recommended
- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics.

Part I. A General Procedures for Transfecting Adherent Cells

Step I. Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 70~80% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection.

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. We recommend using complete serum/antibiotics-containing medium as a starting point. For maximal efficiency and lower cytotoxicity, perform transfection on cells with high density. We recommend transfecting on cells with ~80% confluency.

Step II. Preparation of PeneFect™/DNA Complex and Transfection Procedures:

For different cell types, the optimal ratio of PeneFect™ (μl):DNA (μg) is around 3:1. We recommend the PeneFect™ (μl):DNA (μg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity. To ensure the optimal size of PeneFect™/DNA complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and PeneFect™ Reagent.

The following protocol is given for transfection in 24-well plates, refer to **Table 1** for transfection in other culture formats. The optimal transfection conditions for a majority of adherent cell lines, as well as a general starting point for optimization are given in the standard protocol described below.

- For each well, add 0.5 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
 - For each well, dilute 0.5 μg of DNA into 25 μl of serum-free DMEM with High Glucose. Gently pipette up and down 3~4 times to mix.
 - For each well, dilute 1.5 μl of PeneFect™ reagent into 25 μl of serum-free DMEM with High Glucose. Gently pipette up and down 3~4 times to mix.
- Note:** Never use Opti-MEM to dilute PeneFect™ reagent and DNA, it contains serum and 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.
 - Incubate for 10~15 minutes at room temperature to allow PeneFect™/DNA complexes to form.
- Note:** Never keep the PeneFect™/DNA complex longer than 20 minutes.
- Add the 50 μl PeneFect™/DNA mixture drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
 - Remove PeneFect™/DNA complex-containing medium and replace with fresh complete serum/antibiotics containing medium 12~18 hours post transfection. **For sensitive cells, to lower cytotoxicity, remove PeneFect™/DNA complex and replace with complete medium 5 hours after transfection.**
 - Check transfection efficiency 24 to 48 hours post transfection.

Table 1. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Culture Medium (ml)	Plasmid DNA (μg)	Diluent Volume (ml)	PeneFect™ Reagent (μl)
48 well plate	0.3	0.25	2 × 0.015	0.75
12 well plate	0.75	0.75	2 × 0.038	2.25
6-well plate	1.0	1	2 × 0.05	3.0
35 mm dish	1.0	1	2 × 0.05	3.0
60 mm dish	2.8	2.5	2 × 0.10	7.5
10 cm dish	5.0	5	2 × 0.25	15
T75 flask	8.0	9 - 18	2 × 0.40	27 - 54
250 ml flask	18	25 - 50	2 × 0.8	75 - 105

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

Part II. Advanced Protocol for Transfecting Hard-To-Transfect Mammalian Cells

Important: The advanced protocol for hard-to-transfect cells is provided only if general protocol gives less than 10% efficiency. **For some primary cells which cannot be trypsinized (like primary neurons), go directly to Step II, skip trypsinization and incubate freshly prepared primary cell pellet with transfection complex.**

Step I. Culturing of Cells Before Transfection:

Cells should be plated at least 24 hours prior to transfection so

Table 2. A Guideline for Optimal Cell Number Per Well in Different Culture Formats

Culture Dishes	Surface Area (cm ²)	Optimal Cell Number
T75 Flask	75	9.6 × 10 ⁶
100 mm Dish	58	7.3 × 10 ⁶
60 mm Dish	21	2.7 × 10 ⁶
35 mm Dish	9.6	1.0 × 10 ⁶
6-well Plate	9.6	1.2 × 10 ⁶
12-well Plate	3.5	0.44 × 10 ⁶
24-well Plate	1.9	0.24 × 10 ⁶
48-well Plate	1.0	0.11 × 10 ⁶
96-well Plate	0.3	0.31 × 10 ⁵

Table 3. Recommended Amounts for Different Culture Vessel Formats

Culture Dishes	Transfection Complex Volume (ml)	Plasmid DNA (µg)	PeneFect™ Reagent (µl)
96-well	0.02	0.2	0.8
48-well	0.04	0.5	2
24-well	0.1	1	4
12-well	0.12	1.2	4.8
6-well	0.2	2	8
35 mm dish	0.2	2	8
60 mm dish	0.5	5	20
10 cm dish	1.0	8	32
T75 flask	1.5	36	144
250 ml flask	2.5	100	400

that the monolayer cell density reaches to the optimal 95~100% confluency at the day of transfection.

Step II. Preparation of Cells in Suspension

The following protocol is given for transfecting hard-to-transfect cells in 6-well plates, refer to **Table 2** for optimal cell number per well per Culture vessels' surface area. The optimal transfection conditions are given in the standard protocol described below.

- Detach the cells with trypsin/EDTA and stop the trypsinization with complete culture medium.

Note: Cells that are difficult to detach may be placed at 37 °C for 5-15 min to facilitate detachment

- Take an aliquot of trypsinized cell suspension and count the cells to determine the cell density.

- Centrifuge the required ~1.0×10⁶ cells per well for 6-well plate at 150×g at room temperature for 10 min.

- Use fine tip pipette to remove supernatant **completely** so that no residual medium covers the cell pellet.

Step III. Preparation and application of Transfection Complex

For most of mammalian cells, the optimal ratio of PeneFect™ (µL):DNA (µg) is 4:1. To ensure the optimal size of complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and PeneFect™ Reagent.

The following protocol is given for transfection in 6-well plates, refer to **Table 3** for transfection in other culture formats.

- For each well of 6-well plate, dilute 2 µg of DNA into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to bottom of the tube.

- For each well of 6-well plate, dilute 8 µl of PeneFect™ reagent into 100 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.

- 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 ~15 minutes at room temperature to allow PeneFect™/DNA transfection complexes to form.

Note: Never keep the transfection complexes longer than 20 minutes

- **Gently** resuspend the cell pellet prepared from **Step II** immediately in the 200 µl transfection complex and incubate at 37 °C for 20 minutes.

- At the end of incubation, add 2.0 ml of pre-warmed fresh complete cell growth medium to cells and plate onto one well of a 6-well plate. Incubate at 37 °C with 5% CO₂.

- Remove transfection complex containing medium **gently** and refill with complete culture medium 8~12 hours after plating.

- Check transfection efficiency 24 to 48 hours post transfection.