SKU # SL100569 Store at 4 °C

PowerFect™ In Vitro DNA and siRNA Transfection Kit (Ver. II)

----- A General Protocol for Transfecting siRNA to Mammalian Cells

☐ 100 µL
☐ 500 µL
☐ 1000 µL

SignaGen® Laboratories
5260 Westview Dr, Suite 100
Frederick MD 21703
FAX. 301-560-4919
TEL. 301-330-5966
Toll Free. 1-(866)-918-6812
Email: info@signagen.com
Web: www.signagen.com

This product is for laboratory research ONLY and not for diagnostic use

Introduction:

Based on our innovative and proprietary lipid-conjugation technology, the PowerFect™ Transfection Kit is a liposome-based DNA and siRNA delivery system formulated using our proprietary pH-Dependent Conformational Change (PDCC) technology to achieve highly efficient, reproducible, and robust gene delivery and gene silencing across a broad range of mammalian cell types.

PowerFect™ Transfection Kit is an exceptionally powerful yet gentle transfection reagent optimized for a wide variety of applications involving plasmid DNA and/or siRNA delivery into mammalian cells. Compared with many leading commercial transfection reagents, PowerFect™ consistently provides superior transfection efficiency with significantly reduced cytotoxicity while offering outstanding cost-effectiveness and reproducibility.

Contents Per Kit:

1. 1 × 1.0 mL PowerFect™ DNA In Vitro Transfection Reagent
2. 1 × 8.0 mL PowerFect™ Transfection Buffer (5×)

A General Protocol for DNA/siRNA Co-Transfection

Step I. Preparation of PowerFect™ Transfection Buffer (1×) Working Solution

PowerFect™ Transfection Buffer is supplied as a sterile 5× concentrated stock solution. To prepare the 1× working solution, dilute one volume of PowerFect™ Transfection Buffer (5×) with four volumes of sterile ddH_2O. The resulting 1× working solution is stable at room temperature for up to 24 months.

Note: *Always store PowerFect™ Transfection Buffer (5×) at room temperature. Refrigeration may cause the appearance of white precipitates. The formation of precipitates does not affect transfection performance. Upon dilution with sterile ddH_2O to generate the 1× working solution, the precipitates will completely dissolve.*

Step II. Cell Seeding

Seed cells approximately 18–24 hours prior to transfection so that the cell density reaches an optimal confluency of approximately ~60 at the time of transfection. Fresh complete culture medium containing serum and antibiotics should be added to the cells approximately 30–60 minutes before transfection.

Note: *High serum concentrations (>5%) and the presence of antibiotics do not inhibit transfection efficiency when using PowerFect™ reagent. We therefore recommend maintaining cells in complete growth medium containing both serum and antibiotics throughout the transfection procedure.*

Table 1. General Guidelines for siRNA Transfection Based on Cell Culture Vessel Format

Culture Dish	Growth Medium (mL)	Transfection Buffer (1x) (µL)	siRNA (pmol) Final 5.0 or **50** nM	PowerFect™ Reagent (µL)
24-well	1	50	5.0 / **50**	1.2 ~ **2.0**
12-well	1.5	75	7.5 / **75**	2.0 ~ **3.3**
6-well	2	100	10 / **100**	2.4 ~ **4.0**
60 mm	5	300	25 / **250**	7.2 ~ **12**
10 cm / Flask 75	10	600	50 / **500**	14 ~ **24**

Step III. siRNA Transfection

A final siRNA concentration of 1–100 nM is recommended. For most adherent cell lines and primary cells, a starting concentration of **5 nM** typically yields effective silencing. For difficult-to-transfect cell types, a concentration of **50 nM** is recommended (indicated in bold/underline in **Table 1**).

The following protocol is described per well of a 6-well plate. For other culture vessel formats, refer to **Table 1**.

1. Add 2.0 mL of fresh complete medium (with serum and antibiotics) to each well 30–60 minutes before transfection.
2. Dilute 10 pmol (for 5 nM final) or 100 pmol (for 50 nM final) siRNA into 100 µL of PowerFect™ Transfection Buffer (1×). Vortex briefly to mix.
   Note: Prepare siRNA stocks at 10 µM; add 1.0 µL (for 5 nM) or 10 µL (for 50 nM) per well accordingly. Use of PowerFect™ Transfection Buffer (1×) as the diluent is essential for optimal silencing.
3. Add 2.4 µL PowerFect™ Reagent (or 4.0 µL for difficult-to-transfect cells). Mix by pipetting up and down gently.
4. Incubate the siRNA–reagent mixture at RT for approximately 15 minutes to allow transfection complex formation.
   Note: Do not extend complex incubation beyond 30 minutes, as this may reduce transfection efficiency.
5. Add the transfection complex dropwise to the cells. Rock the plate gently and return the plate to the CO_2 incubator.
6. If required, replace the transfection medium with fresh complete growth medium approximately 5 hours post-transfection.
7. Assess gene silencing by qRT-PCR, Western blot, or functional assay 48–72 hours post-transfection.

Storage: PowerFect™ siRNA Transfection Reagent is stable for up to **24 months at 4° C**. PowerFect™ Transfection Buffer (5 x) and (1x) must be stored at RT. The product is shipped at ambient temperature.

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PowerFect™ siRNA Transfection Reagent

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Add fresh complete growth medium 30–60 minutes before transfection.
Note: PowerFect™ Reagent performs equivalently in the presence of serum and antibiotics; serum-free media substitution is not required.

Step 3. DNA/siRNA Co-transfection

For co-transfection, use 0.3–0.5 µg plasmid DNA and 1–20 nM siRNA per well of a 6-well plate. A recommended starting condition is 0.5 µg DNA and 20 pmol siRNA (10 nM final concentration) per well, which yields satisfactory knockdown for most cell lines.

The following protocol applies per well of a 6-well plate. For other culture formats, refer to **Table 2**.

1. Add 1.0 mL of fresh complete medium (with serum and antibiotics) to each well 30–60 minutes before transfection.
2. Combine 0.5 µg plasmid DNA and 10 pmol siRNA in 100 µL of PowerFect™ Transfection Buffer (1×). Mix gently by pipetting.
   Note: Prepare siRNA stocks at 10 µM; add 1.0 µL per well to achieve a 10 nM final concentration. Use of PowerFect™ Transfection Buffer (1×) for dilution is critical for co-transfection efficiency.
3. Immediately add 3 µL PowerFect™ Reagent and mix by pipetting up and down.
4. Incubate at RT for approximately 15 minutes to allow ternary complex formation.
   Note: Do not exceed 30 minutes of complex incubation.
5. Add the transfection complex dropwise to the cells. Rock the plate gently and return to the CO_2 incubator.
6. Replace the transfection medium with fresh complete growth medium approximately 5 hours post-transfection, if required.
7. Analyze knockdown efficiency 48–72 hours post-transfection by appropriate molecular or functional assays.

Table 2. General Guidelines for DNA and siRNA Co-Transfection According to Cell Culture Vessel Format

Culture Dish	Growth Medium (mL)	Transfection Buffer (1x) (µL)	Plasmid DNA (µg)	siRNA (pmol) Final 10 nM	PowerFect Reagent (µL)
24-well	1.0	50	0.25	10	1.5
12-well	1.5	75	0.375	15	2.25
6-well	2.0	100	0.5	20	3
60 mm	5.0	300	1.5	50	7.5
10 cm / flask 75	10	600	4.0	100	15