

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

----- A General Protocol for Transfecting Mammalian Cell

- 100 µl
- 500 µl
- 1000 µl



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

Introduction:

Based on our innovative and proprietary lipid-conjugation technology, PowerFect™ Transfection Kit is a liposome based DNA & siRNA delivery tool which was formulated with our proprietary pH Dependent Conformational Change (PDCC) technology to give efficient and reproducible gene knockdown on variety of mammalian cells. PowerFect™ Transfection Kit is the most powerful yet very gentle gene delivery tool for a variety of applications including plasmid DNA and/ or siRNA for most of mammalian cell types. Compared with leading products in the market, PowerFect™ is more cost-effective and always provides higher transfection efficiency with less cytotoxicity.

Contents Per Kit:

- 1x 1.0 ml of PowerFect™ DNA In Vitro Transfection Reagent
- 1x 8.0 ml of PowerFect™ Transfection Buffer (5x)

Important Guidelines for Transfection:

- PowerFect™ reagent was formulated for DNA and siRNA transfection. The following standard protocol is given for DNA/siRNA co-transfection to mammalian cells. For a protocol of DNA and siRNA transfections, please email us at info@signagen.com
- For better efficiency, choosing PowerFect™ Transfection Buffer (1x) is a must.
- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics.

A General Protocol for DNA/siRNA Co-transfection.

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

PowerFect™ Transfection Buffer (5x) is provided as 5x concentrated stock solution. To make working solution, dilute one part of the stock solution with 4 parts of ddH₂O. The PowerFect™ Transfection Buffer (1x) working solution is stable at RT for 24 months.

Note: Always keep PowerFect™ Transfection Buffer (5x) at RT. If refrigerated, white precipitates may appear. It won't affect the transfection efficiency. After dilution with 4 parts of ddH₂O to make PowerFect™ Transfection Buffer (1x) working solution, the white precipitates will disappear.

Step II. Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 60-70% 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 do NOT have inhibitory effect on transfection efficiency. We recommend using complete serum/antibiotics-containing medium to grow the cells during transfection.

Step III. Preparation of PowerFect™ -DNA/siRNA Complex and Transfection Procedures:

For DNA/siRNA co-transfection, we recommend using 1.0 µg DNA and 10 to 80 nM siRNA per well in a 6-well plate. The following protocol is given per well of 6-well plate. For other culture formats, please refer to [Table 1](#).

- For each well, dilute 1 µg of DNA and 20 to 160 pmoles siRNA into 200 µl of PowerFect™ Transfection Buffer (1x) prepared from [Step I](#). Mix by vortexing.
 - Add 4 µl of PowerFect™ reagent, vortex briefly to mix.
 - Incubate for ~10 min at RT to allow PowerFect™/DNA/siRNA complex to form.
- Note:** Never keep the PowerFect™/DNA/siRNA complex longer than 20 min.
- Add the PowerFect™/DNA/siRNA transfection mix to the cells in serum containing medium drop wisely.
 - Swirl plate gently to homogenize.
 - Check transfection efficiency 24 to 72 hours post transfection. 48-72 hours usually give better efficiency.

Table 1. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Culture Medium (ml)	Plasmid DNA (µg)	siRNA Final 10-80 nM (pmoles)	PowerFect™ Transfection Buffer (1x) (µL)	PowerFect™ Reagent (µL)
24-well	0.25	0.25	2.5-20	25	1-1.5
12-well	0.5	0.5	5-40	50	2-3
6-well	2	1.0	10-80	200	4-6
35 mm dish	2	1.0	20-160	200	4-6
60 mm dish	4	2.0	40-320	400	8-12
10 cm/T75	10	5	100-800	800	20-30
15 cm/T175	20	10	200-1600	1600	40-60

Storage: PowerFect™ Reagent is stable for up to 12 months at +4 °C after receipt