Cat # SL100569 Store at 4 °C

PowerFect™ siRNA Transfection Reagent

----- A General protocol for transfecting mammalian cells

- ☐ 100 µl
- ☐ 500 µl
- ☐ 1000 µl

SignaGen® Laboratories
10075 Tyler Place, Suite 19
Ijamsville, MD 21754
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:

PowerFect™ Reagent is liposome based 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™ Reagent have been validated to effectively and reproducibly transfect single siRNA and co-transfect DNA/siRNA to variety of mammalian cells.

Important Guidelines for Transfection:

- PowerFect™ transfection reagent was formulated as a siRNA delivery tool. This protocol gives procedures for co-transfecting siRNA/DNA and for transfecting siRNA to mammalian cells.
- For maximum gene silencing, we recommend using PowerFect™ Transfection Buffer to dilute siRNA/DNA and PowerFect™ Reagent. Never use pyruvate or serum containing mediums like DMEM or Opti-MEM to dilute siRNA/DNA and PowerFect™ reagent.

1. DNA & siRNA Co-transfection

1.1 Preparation of Working Solution of PowerFect™ Transfection Buffer:

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 into a sterile bottle. The working solution is table at 4 ^{0}C~RT for 12 months.

1.2 Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~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: PowerFect™ reagent is NOT interfered by serum and antibiotics, therefore serum and antibiotic containing medium can be used during the entire experiment.

Table 1. A Guideline for DNA & siRNA Co-transfection Per Cell Culture Vessel

Culture Dish	Growth Medium (ml)	Diluent Volume (µL)	Plasmid DNA (µg)	siRNA Final 5.0 nM (pmoles)	PowerFect™ Reagent (µL)
24-well	0.5	50	0.25	2.5	1.5
12-well	0.75	75	0.38	3.75	2.25
6-well	1.0	100	0.5	5	3
60 mm	3.0	300	1.5	15	9
10 cm /flask 75	8.0	800	4.0	40	24

1.3 DNA & siRNA co-transfection protocol:

For DNA/siRNA co-transfection experiment, we recommend using 0.5~1.0 µg DNA and 5 ~ 50 nM siRNA per well in a 6-well plate. As a starting point, we recommend using 0.5 µg DNA and 5.0 pmoles siRNA (final concentration 5 nM) per well of a 6-well plate which usually give satisfactory silencing effect.

The following conditions are given per well of a 6 well plate. For other culture format, please refer to **Table 1**.

- For each well, add 1.0 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- Dilute 0.5 µg DNA and 5 pmoles siRNA (final 5 nM) into 100 µl working solution of PowerFect™ transfection buffer. Mix by pipetting up and down.

  Note: For optimal transfection efficiency and maximum knockdown result, use PowerFect™ transfection buffer to dilute siRNA/DNA and PowerFect™ reagent. Pyruvate and serum interfere formation of the transfection complex. So never use mediums like DMEM which may contain sodium pyruvate and Opti-MEM which contains serum.

  We strongly suggest preparing siRNA stock solution at 5.0 µM, so add 2.0 µl siRNA stock solution per well of 6-well plate to make final 10 nM of siRNA.
- Add 3 µl PowerFect™ reagent immediately followed by pipetting up and down to mix.
- Incubate for ~15 min at RT to let transfection complex form. **Note: Never keep the transfection complex longer than 30 minutes**.
- Add the transfection complex to the cells drop wise.
- Gently rock the plate back and forth and return the plate to the incubator.
- Check the knockdown effect 24~48 hours post transfection. Replace transfection medium by cell growth medium ~5 hours after transfection if necessary.

2. siRNA Transfection

2.1 Preparation of Working Solution of PowerFect™ Transfection Buffer:

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 ddH_2O into a sterile bottle. The working solution is table at 4 ^{0}C~RT for 12 months.

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2.2 Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal ~50% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection.

2.3 siRNA Transfection Protocol:

For optimal siRNA-mediated silencing, we recommend using 5~50 nM siRNA. As a starting point, we recommend using 20 nM siRNA which usually gives satisfactory knockdown effect.

The following conditions are given per well in a 6 well plate. For other culture format, please refer to **Table 2**.

- For each well, add 1.0 ml of complete medium with serum and antibiotics freshly 30 ~ 60 minutes before transfection.
- Dilute 20 pmoles siRNA (final concentration of 20 nM per well) into 100 µl working solution of PowerFect™ Transfection Buffer. Mix by pipetting up and down.

  Note: For optimal transfection efficiency and maximum knockdown result, use PowerFect™ transfection buffer to dilute siRNA and PowerFect™ reagent.
  We strongly suggest preparing siRNA stock solution at 10 µM, so add 2.0 µl siRNA stock solution per well of 6-well plate to make final 20 nM of siRNA.

- Add 2.4 µl PowerFect™ reagent followed pipetting up and down.
- Incubate for ~15 min at RT to let transfection complex form.

  Note: Never keep the complex longer than 30 minutes.
- Add the transfection mix to the cells drop wise.
- Gently rock the plate back and forth and return the plate to the incubator.
- Check knockdown effect 24~48 hours post transfection. Replace transfection medium by cell growth medium ~5 hours after transfection if necessary.

Table 2. A Guideline for siRNA Transfection Per Cell Culture Vessel

Culture Dish	Growth Medium (ml)	Transfection Buffer (µL)	siRNA (pmoles) 20 nM Final	PowerFect ™ reagent (µL)
24-well	0.5	50	10	1.2
12-well	0.75	75	15	1.8
6-well	1.0	100	20	2.4
60 mm	3.0	300	60	7.2
10 cm /flask 75	8.0	800	160	20

Storage: PowerFect™ siRNA Transfection Reagent is stable for up to 12 months at 4 °C. This item shipped at ambient temperature