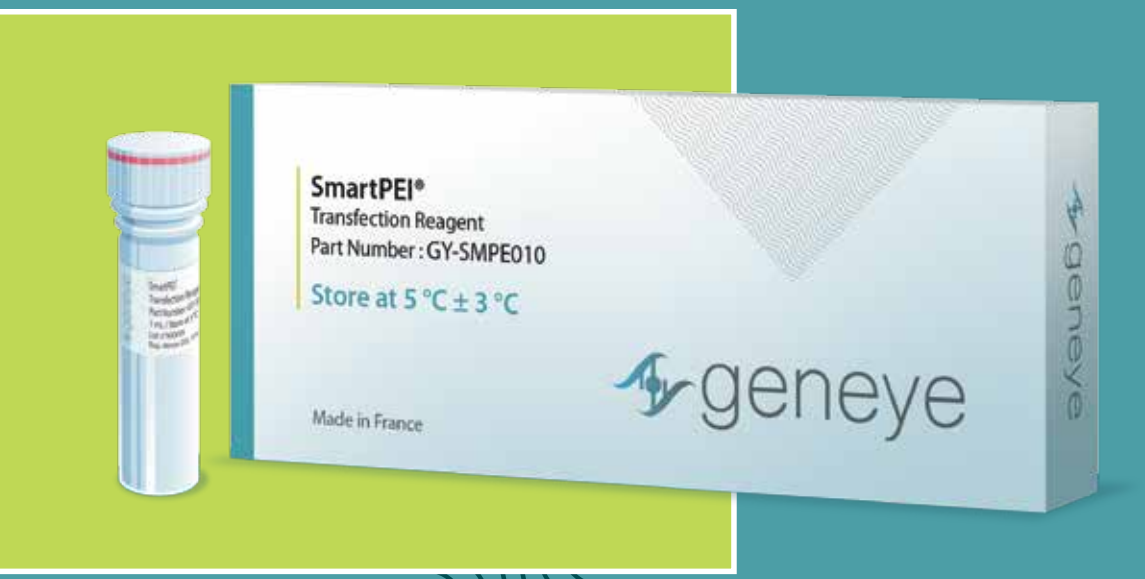
Smart PEI®

TRANSFECTION REAGENT

Part Number: GY-SMPE010

Smart PEI®

Instructions for Use



For research use only.
Not for use in humans.

geneye

SmartPEI®

TRANSFECTION REAGENT

1. Intended Use

SmartPEI® reagent is a low toxicity, serum-compatible transfection reagent enabling robust, effective and reproducible DNA transfection into mammalian cells. SmartPEI reagent, mainly composed of a linear polyethylenimine, provides better in vitro transfection than common cationic lipids or polymers. Main applications include High Throughput Screening (HTS) due to its high reproducibility.

SmartPEI reagent is compatible with a broad range of cell lines and primary cells. Contact your local Geneye representative for more information.

SmartPEI transfection reagent is stable in the presence of serum therefore you may use serum-containing media.

Up to approximately 5 000 to 10 000 transfections in 96-well plates or 2 000 to 4 000 transfections in 24-well plates can be performed with 1 mL of SmartPEI reagent.

This product must not be used for any other purpose.

Geneye recommends that users are appropriately trained before using this product.

Shipment at room temperature.

Store the SmartPEI Transfection Reagent at refrigerated temperature (5 °C ± 3 °C).

Expiration date: See date printed on the tube. Typically, shelf life is one year.

2. Safety Informations

The SmartPEI transfection reagent is non-hazardous. However, all disposable materials must be discarded according to appropriate waste procedures used in the laboratory.

This product must only be used by trained personnel in accordance with good laboratory practice.

3. Inspection on Receipt

Upon receipt, user must check that the product is the one required for their application.

In addition, Geneye recommends a visual inspection of the item as received. Should there be any indication of product or packaging damage, the item should not be used and should be returned to your local Geneye representative.

4. Principle

With SmartPEI transfection reagent, DNA molecules are compacted into positively charged complexes that can be internalized by endocytosis following binding of these complexes with anionic proteoglycans on the cell surface.

SmartPEI reagent also prevents DNA complexes to be lyzed while inside endosomes by regulating pH and subsequently leading to the disruption of endosomes, release of DNA complexes into the cytoplasm and finally allowing the nuclear delivery for subsequent transcription.

5. Pack Contents

The pack contains:

- Microtube(s) containing 1 mL of SmartPEI transfection reagent
- 1 Instructions for Use document

6. Consumables Required but Not Included

- Normal saline solution (9 g/L)
- Cultured cells
- Culture vessel
- Cell culture medium
- Sterile microtube or tube
- Micropipette and tips, pipet and pipet-aid

7. Operating Mode

SmartPEI transfection reagent can be used for:

- Forward (transient) transfection of adherent (described here) and suspended cells (see Protocol Guidelines)
- Stable transfection of adherent cells (See Protocol Guidelines)
- Reverse transfection (recommended for High Throughput Screening) (See Protocol Guidelines)
- Batch transfection (See Protocol Guidelines)

1. FORWARD TRANSFECTION PROTOCOL FOR ADHERENT CELLS (TRANSIENT)

This protocol is recommended for routine experiments.

A. CELL CULTURE AND SEEDING

In this protocol, after proceeding to cells, the transfection complexes are added the following day to the cells in serum-containing medium.

Ideally, use 50-70% confluent cells at the day of transfection to reach optimal transfection efficiency.

- For 96-well plate: 24 hours prior transfection: seed 10 000 to 17 000 cells/well
- Incubate cell cultures
- The next morning before transfection: change culture medium (add 0.2 mL of medium per well)

The protocol described here is a standard protocol for cell seeding in a 96-well plate.
Use Table 1 for other culture formats.

Table 1. Recommended number of adherent cells to seed per culture format.

Culture format	Number of adherent cells to seed	Surface area per well (cm^2)	Volume of medium per well, dish or flask (mL)
384-well plate	5 000 - 10 000	0.056	0.05 - 0.1
96-well plate	10 000 - 17 000	0.3	0.1 - 0.2
48-well plate	25 000 - 50 000	1	0.25 - 0.5
24-well plate	50 000 - 100 000	1.9	0.5 - 1
12-well plate	80 000 - 200 000	3.8	1 - 2
6-well plate/35 mm	200 000 - 400 000	9.4	2 - 4
6 cm dish/25 cm^2 flask	400 000 - 800 000	25-28	5 - 10
10 cm dish/75 cm^2 flask	2 000 000 - 4 000 000	75-78.5	10 - 15
14 cm dish/175 cm^2 flask	4 000 000 - 8 000 000	153 - 175	20 - 30

B. DNA COMPLEXES PREPARATION AND TRANSFECTION

1. For 96-well plate: In a sterile microtube, for each well of the culture plate, dilute 0.25 µg of DNA per well in saline solution (9 g/L). Final volume is 10 µL per well.
2. Vortex gently and spin down briefly.
3. Vortex SmartPEI reagent for 5 sec and spin down.
4. In a separate microtube, for each well, dilute 0.5 µL of SmartPEI reagent in 9.5 µL of saline solution (9 g/L). Final volume is 10 µL per well.

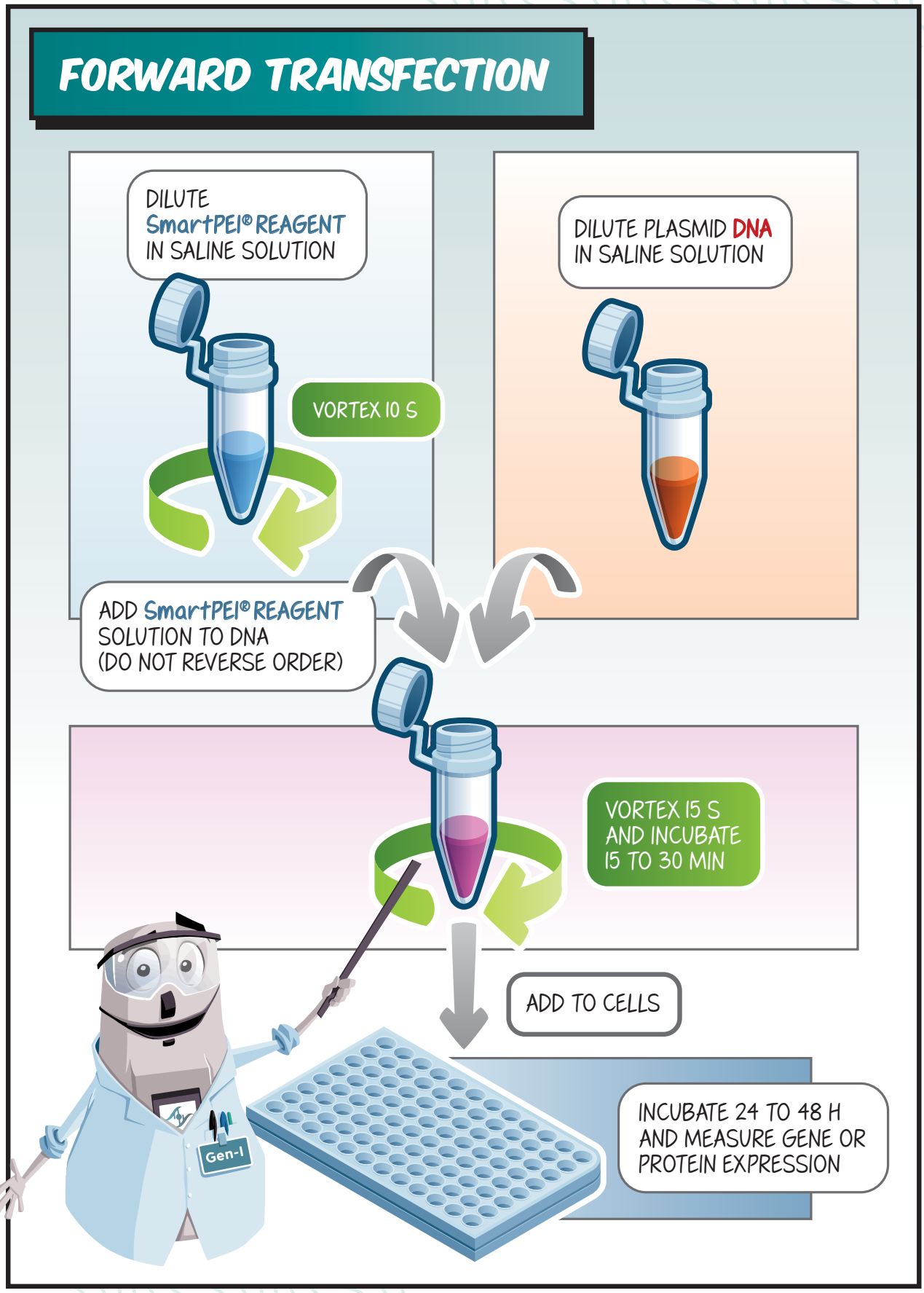
Note: Geneye recommends using a standard ratio of 2 µL of SmartPEI reagent per µg of DNA. This volume can be adjusted from 1 to 4 µL/µg of DNA depending on the cell line.

5. Vortex gently and spin down briefly.
6. Transfer the SmartPEI solution prepared to the DNA solution all at once. Completing transfer the other way can impact transfection efficiency.
7. Vortex the solution, spin down briefly and incubate at room temperature for 15 to 30 minutes.
8. In the 96-well culture plate, distribute dropwise 20 µL per well of SmartPEI reagent /DNA mixture to the cells in 0.2 mL of serum-containing medium and homogenize gently.
9. Incubate cell cultures.
10. Test transfection efficiency with reporter gene assay 24 to 48 h after transfection.

The protocol described here is a standard protocol for transfection in a 96-well plate. Use Table 2 for other culture formats.

Table 2. Guidelines for transfection complex preparation per culture formats

Culture format	DNA quantity per well (µg)	Volume of SmartPEI reagent per well (µL)	Final volume of saline solution for DNA and SmartPEI reagent per well (µL)	Final volume of complexes added per well (µL)
384-well plate	0.1	0.2	5	10
96-well plate	0.25	0.5	10	20
48-well plate	0.5	1	25	50
24-well plate	1	2	50	100
12-well plate	2	4	50	100
6-well plate/35 mm	3	6	100	200
6 cm dish/25 cm^2 flask	5	10	250	500
10 cm dish/75 cm^2 flask	10 - 20	20 - 40	250	500
14 cm dish/175 cm^2 flask	20 - 30	40 - 60	500	1000


FORWARD TRANSFECTION
DILUTE SmartPEI® REAGENT IN SALINE SOLUTION
VORTEX 10 S
DILUTE PLASMID DNA IN SALINE SOLUTION
ADD SmartPEI® REAGENT SOLUTION TO DNA (DO NOT REVERSE ORDER)
VORTEX 15 S AND INCUBATE 15 TO 30 MIN
ADD TO CELLS
INCUBATE 24 TO 48 H AND MEASURE GENE OR PROTEIN EXPRESSION
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8. Troubleshooting

PROBLEM	SUGGESTIONS
Low transfection efficiency	• Use serum-containing culture medium. • Check confluence at the time of transfection, recommend 50%-70%. • Optimize the transfected DNA quantity. • Decrease the volume of culture medium per well. • If compatible with cell line, centrifuge the plates for 5 min at 180 *g* after addition of transfection complexes. • Optimize the SmartPEI reagent/DNA ratio, up to 1:4. • Check purity of plasmid DNA preparation (OD 260/280 > 0.8). • Use a 0.3 to 1 µg/µL DNA preparation. • Use a positive control (plasmid including a common reporter gene, e.g. Luciferase).
Cellular toxicity	• At constant SmartPEI reagent/DNA ratio, reduce the quantity of DNA. • Reduce SmartPEI reagent/DNA ratio if above 2 µL of SmartPEI reagent per 1 µg of DNA. • Change medium 4h or 2h after transfection. • If the expressed transfected protein is toxic for cells, reduce the quantity of DNA used during transfection. • Use an endotoxin-free DNA preparation. • Use a negative control (e.g. a SmartPEI reagent/non-coding plasmid complex).

9. Disposal

This product should be disposed of in line with local and federal requirements for the materials of construction of the article and taking into consideration any contaminants or reagents present with the article as a result of the experiment undertaken in use.

10. Performance

Each batch of SmartPEI transfection reagent is tested for transfection on cells.

Visit us on the Web at geneye.com - contact us at info@geneye.com

Because of technological developments related to the products, systems, and/or services described herein, the data and procedures are subject to change without notice.

Please consult your Geneye representative or visit **www.geneye.com** to verify that this information remains valid.

IFUGYSMPEI December 2018