**PULSin™: a potent reagent for protein delivery to eukaryotic cells**

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**Abstract**

PULSin™ is a potent reagent for protein delivery to the cytoplasm of live eukaryotic cells. PULSin™ forms non-covalent complexes with proteins or antibodies. These complexes are internalized via anionic cell-adhesion receptors present on virtually all cells and are released into the cytoplasm where they disassemble. The native protein can thus diffuse throughout the cytoplasm and exert its function or proceed to its target organelle. PULSin™ delivers proteins to a large variety of live eukaryotic cells, including primary and suspension cells, without noticeable side effects. PULSin™-mediated antibody delivery is a convenient method for immunostaining of live cells and for protein interference studies.

**Introduction**

Intracellular delivery of functional biomolecules such as proteins or antibodies using synthetic carriers is an alternative to nucleic acid transfection where proteins are produced in situ after expression of the transgene. Nuclear transport is recognized as a major obstacle for gene transfection and subsequent expression whereas it does not hinder protein delivery. Indeed most soluble proteins can exert their biological function if delivered to the cytoplasm where diffusion and signal-dependent localization will address them to their final destination. The major hurdles for efficient protein delivery are therefore crossing the plasma membrane and release in the cytoplasm.

**Figure 2: Kinetics of intracellular R-PE delivery into human primary fibroblasts**

One µg of R-PE was diluted in 100 µl Hepes buffer (20 mM, pH 7.4), and added directly (a) or after 15 minutes complex formation with 4 µl of PULSin™ (b-d) onto human primary fibroblasts in a 24-well plate. Intracellular protein delivery was analyzed after 4 hours (b), 8 hours (c) or 20 hours (d) after addition of R-PE/PULSin™ complexes onto cells. White arrows indicate intracellular protein/PULSin™ vesicles.
Amino-acid sequences and posttranslational modifications contribute to protein diversity. In terms of properties, proteins differ by their molecular weight, isoelectric point, polarity and hydrophobicity. While some are able to enter cells spontaneously, the majority of proteins requires alternative delivery methods which take into account their natural diversity.

Physical, viral and chemical methods have been employed to introduce proteins into mammalian cells. Methods such as electroporation or microinjection which were first developed for gene transfection have been adapted to intracellular protein delivery. The practice of these methods however requires technical skills. Furthermore, electroporation has a narrow window of efficiency and microinjection is limited to a few numbers of cells per experiment. Viral methods are mainly used for vaccination.

Chemical methods are either based on protein encapsulation into liposomes or on a complex formation/aggregation mechanism acting via endocytosis. This process involves interaction of polypeptide complexes with the plasma membrane or membrane-associated receptors. In a second step, energy-dependent uptake leads to the formation of intracellular vesicles. Finally, the carried protein needs to be released from the vesicles into the cytoplasm.

Various carriers including cationic peptides, polymers, and lipids have been developed to transfer proteins across the plasma membrane. Short cationic peptides (<30 residues), named cell-penetrating peptides (CPPs) or translocating peptides from homeobox or viral proteins were initially used. Subsequently, comparable delivery results were obtained with oligoysine or arginine sequences.

The mechanism of translocation was thought to be receptor- and energy-independent. In fact, misinterpretation of cell internalization data and in some cases cell fixation artefacts have raised doubts regarding the efficiency and the use of CPPs as intracellular carriers. This concern also holds for fusion approaches where the protein to be delivered is covalently linked to a cargo. Recent results show that CPPs cargoes are found at the cell surface and sequestered within intracellular vacuoles. Cationic lipid carriers are a step ahead. Their strength lies in a positively-charged headgroup and a lipophilic tail that allow complex formation with proteins via electrostatic and hydrophobic interactions.

The mechanism of delivery is similar to that of gene delivery. The interaction between PULSin™ and R-phycocerythrin (R-PE), a 240-kDa autofluorescent protein, was analyzed under native conditions using agarose gel electrophoresis (figure 1). R-PE (isoelectric point: 5.5) migrated towards the cathode but was retained in the well when incubated for 1.5 minutes at room temperature with PULSin™ prior to electrophoresis.

At Polyplus-transfection, we have tested a wide range of cationic lipid formulations with several types of proteins on many cell types. Finally, we propose PULSin™, an aqueous formulation of small and stable cationic liposomes characterized by a mean size of 100 nm and a surface potential of +50 mV.

Due to the strong charge and lipophilic property, PULSin™ binds to most proteins. The interaction between PULSin™ and R-phycocerythrin (R-PE), a 240-kDa autofluorescent protein, was analyzed under native conditions using agarose gel electrophoresis (figure 1). R-PE (isoelectric point: 5.5) migrated towards the cathode but was retained in the well when incubated for 1.5 minutes at room temperature with PULSin™ prior to electrophoresis.
intracellular vesicles. Only a small fraction of signal associated to mostly observed in the cytoplasm leaving 2d), the fluorescent signal was maximal and the endosomes. Finally, after 20 hours (figure 2c), the intracellular fluorescence was enhanced as more protein was released from (figure 2d), either in the endosomes or a speckled fluorescent signal (white arrows observed after 4 hours incubation (figure 2b). Cells, intracellular delivery of R-PE was already due to the size of the R-PE which is larger than nuclear pores. A fraction of R-PE was observed in the nuclear compartment signal throughout the cytoplasm. No signal was observed in the nuclear compartment due to the size of the R-PE which is larger than nuclear pores. A fraction of R-PE was still observed as complexed with PULSin™ as a speckled fluorescent signal (white arrows in figure 2b-d), either in the endosomes or in the cytoplasm. After 8 hours incubation (figure 2c), the intracellular fluorescence was enhanced as more protein was released from the endosomes. Finally, after 20 hours (figure 2d), the fluorescent signal was maximal and mostly observed in the cytoplasm leaving only a small fraction of signal associated to intracellular vesicles.

**Mechanism of protein delivery using PULSin™**

We further analyzed the intracellular delivery mechanism in order to determine whether this process is energy-dependent or not. The first 4 hours are critical for the interaction of protein/PULSin™ complexes to the cells and subsequent endocytosis. When the cells were incubated at 4°C, the protein/PULSin™ complexes were already observed after 4 hours incubation (figure 2b). Free R-PE appeared as a diffuse fluorescent signal throughout the cytoplasm. No signal was observed in the nuclear compartment due to the size of the R-PE which is larger than nuclear pores. A fraction of R-PE was still observed as complexed with PULSin™ as a speckled fluorescent signal (white arrows in figure 2b-d), either in the endosomes or in the cytoplasm. After 8 hours incubation (figure 2c), the intracellular fluorescence was enhanced as more protein was released from the endosomes. Finally, after 20 hours (figure 2d), the fluorescent signal was maximal and mostly observed in the cytoplasm leaving only a small fraction of signal associated to intracellular vesicles.

Delivery of R-PE, a red fluorescent protein, was assed using a fluorescence microscope. As shown in figure 2a, the addition of R-PE (1 µg) alone to human primary fibroblasts did not show a fluorescent signal. When R-PE was complexed with PULSin™ and added to the cells, intracellular delivery of R-PE was already observed after 4 hours incubation (figure 2b). Free R-PE appeared as a diffuse fluorescent signal in the cytoplasm. No signal was observed in the nuclear compartment due to the size of the R-PE which is larger than nuclear pores. A fraction of R-PE was still observed as complexed with PULSin™ as a speckled fluorescent signal (white arrows in figure 2b-d), either in the endosomes or in the cytoplasm. After 8 hours incubation (figure 2c), the intracellular fluorescence was enhanced as more protein was released from the endosomes. Finally, after 20 hours (figure 2d), the fluorescent signal was maximal and mostly observed in the cytoplasm leaving only a small fraction of signal associated to intracellular vesicles.

**Mechanism of protein delivery using PULSin™**

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Even after a prolonged incubation at 37°C (16 additional hours), no protein delivery was observed in the cells initially incubated at 4°C (figure 3a, upper right), when compared to cells incubated at 37°C for 20 hours showing a bright diffuse fluorescent signal in the cytoplasm (figure 3a, lower right). These results strongly suggest that the incubation temperature influences the fate of the complexes.

In order to quantify these observations, we have analyzed by fluorescence-activated cell sorting (FACS) the amount of intracellular fluorescent protein after incubation at 4°C and 37°C. R-PE (0.5, 1 or 2 µg) was incubated with 4 µl of PULSin™ and added to HeLa cells (figure 3b). Analysis of the overall delivery efficiency was determined after 20 hours incubation. Interestingly, the efficiency strongly depended on the incubation temperature, with almost no delivery at 4°C. As expected, no delivery was observed when R-PE alone was added as a control. This set of experiments confirmed that protein delivery mediated by PULSin™ is an energy-dependent process likely to occur via endocytosis.

We thus propose a general mechanism for protein delivery mediated by PULSin™ that is reminiscent of gene delivery. As depicted in figure 4, positively-charged complexes formed between the protein and PULSin™ interact with negatively charged heparan sulfate proteoglycans (HSPG) at the cell surface. Cooperative electrostatic binding induces HSPG aggregation which in turn triggers endocytosis of the complexes. PULSin™ promotes release of the complexes from the endosomes, allowing neutralization of the cationic lipid by anionic phosphatidyserine from the external leaflet of the endosome, hence complex dissociation. Finally, the native protein is able to diffuse within the cytoplasm to reach its target organelle or compartment. This process is non-toxic and allows delivery of large amounts of functional protein.

**PULSin™ protocol**

From an experimental point of view, PULSin™ is a ready-to-use protein delivery reagent with a fast and simple protocol (figure 5). Cells are plated the day before protein delivery in order to reach approximately 80% confluency when the complexes are added to the cells. For one well of a 24-well plate, 1 µg of purified protein delivery or incubate at 37°C for a longer period.

1. Dilute 1 µg of protein in 100 µl Hepes 20 mM, pH 7.0.
2. Add 4 µl of PULSin™.
3. Incubate for 15 min at RT.
4. Rinse cells with PBS and add 900 µl of culture medium w/o serum per well.
5. Add solution of protein/PULSin™ complexes directly to the cells and incubate for 4 hours at 37°C.
6. Replace medium with fresh complete medium (with serum). Analyze protein delivery or incubate at 37°C for a longer period.
protein is diluted in 100 µl of 20 mM Hepes buffer (supplied with PULSin™) in a 1.5 ml microcentrifuge tube, under sterile conditions. Four µl of PULSin™ are added to the protein solution. After a brief homogenization with a vortex and a short centrifugation, the protein/PULSin™ mix is incubated for 1.5 minutes at room temperature. The cells are washed with 1 ml of PBS and 900 µl of basal culture medium without serum are added to the well. After addition of 100 µl protein/PULSin™ solution onto each well, the plate is gently mixed and incubated for 4 hours at 37°C. The solution is then transferred to fresh complete medium (containing serum).

Protein delivery or activity can be analyzed 1 ml of PBS and 900 µl of basal culture medium without serum are added to the well.

After incubation, the cells are washed with PBS and the protein/PULSin™ complex is collected. The cells are then incubated for 15 minutes at 4°C. The optimized volume of PULSin™ allows the cells to be washed with fresh complete medium (containing serum). The cells are centrifuged and resuspended in fresh complete medium (containing serum).

Protein delivery optimization

As shown for the R-PE delivery mediated by PULSin™ into human primary fibroblasts (figure 2), protein delivery is a variable, time-dependent phenomenon. Each experiment carried out on a specific cell type with a protein of interest should be analyzed in terms of kinetics.

The amount of protein to be used per well has also to be determined for each protein and each cell type. For R-PE delivery, the optimal quantity per well (in a 24-well plate) is 1 µg of protein complexed with 4 µl of PULSin™, as illustrated in figure 2. The efficiency of delivery is clearly dependent on the amount of protein used. As illustrated in the figure 6, a 2-fold increase or decrease of this optimal amount greatly influences the level of fluorescent signal observed in 3T3 L1 cells 16 hours after delivery (figure 6a). A low diffuse fluorescence was visible with 0.5 µg of R-PE, while with 2 µg the intracellular signal was saturated.

The overall efficiency of protein delivery was also evaluated by FACS (figure 6b). Delivery of BSA, a non-fluorescent protein, defined the background fluorescence of the cell. The percentage of gated cells times their mean fluorescence intensity linearly increased as a function of the amount of fluorescent protein used to form the complexes.

Another parameter to optimize when performing protein delivery experiments is the volume of PULSin™ reagent to be added when preparing the complexes. The optimized volume of PULSin™ to deliver 1 µg of R-PE in a 24-well plate is 4 µl. Using only 2 µl of PULSin™ results in lower efficiency (data not shown).

PULSin™ efficiency in various cell types

As illustrated in figure 7, PULSin™ is able to deliver proteins to a wide variety of cells including adherent cell lines such as epithelial-, fibroblast-, macrophage-like cells as well as primary cells and non-adherent cells. The efficiency varies depending on the cell type, but in most cells high level of protein delivery can be obtained (e.g. up to 95% for NIH-3T3 cells). For even hard-to-transfect suspension cells, such as Jurkat or K-562 cells, we obtained approximately 60-80% and 25% protein delivery, respectively. A specific protocol for fragile cells is also available.

Applications of PULSin™-mediated protein delivery

Delivery of antibodies to live cells (non-permeabilized) presents a wide range of interesting applications. Antibodies are able to recognize proteins in cells and/or block protein function (so called protein interference) or follow intracellular trafficking. DNA transfection in eukaryotic cells is not generally used to produce antibodies in situ, mainly because of inappropriate folding in the cytoplasm (scFv antibodies are an exception). Thus, intracellular delivery of antibodies using PULSin™ is a well-suited solution for such applications.

As depicted in figure 8, FITC-anti-α-tubulin antibody (monoclonal mouse IgG1) was successfully delivered to live HeLa cells using PULSin™. As a negative control, the antibody was added onto cells (figure 8a) and no fluorescent signal was observed. Without PULSin™, antibodies fail to enter the live cells. In the presence of PULSin™, a green fluorescent signal was observed and essentially associated with the actin cytoskeleton.

We also quantified antibody delivery in HeLa cells using cell sorting. The fluorescent (FITC) antibody was delivered to over 60% of gated cells (figure 8b).

Finally, as illustrated in figure 8, PULSin™ delivers functional primary antibodies that are able to reach their target in the cell. Remarkably, unlike immunocytochemistry, delivery of antibodies using PULSin™ allows intracellular staining of live cells, i.e. cells that are not fixed or permeabilized. Keeping in
Adherent cell lines

- 3T3 L1: 60-80%
- A549: 80%
- BHK-21: 30-40%
- CaSki: 80-90%
- CHO: 80-90%
- CV-1: 50%
- HEK-293: 45-55%
- HeLa: 80-90%
- MCF-7: 60%
- MLE 15: 60-75%
- NIH-3T3: 90-98%
- RAW 264.7: 40-50%
- SiHa: 60-70%

Adherent primary cells

- Human keratinocytes: 55-70%
- Human preadipocytes: 60-75%
- Human fibroblasts: 60-70%
- Human hepatocytes: 50-70%

Suspension cell lines

- Jurkat: 60-80%
- K-562: 20-30%
- HEK-293: 30-40%
- THP-1: 10%

Figure 7: Efficiency of intracellular R-PE delivery: FITC-anti-α-tubulin antibody delivery. FITC-anti-α-tubulin was carried out in the absence (-) or presence (+) of PULSin™-mediated intracellular delivery of anti-α-tubulin FITC antibody to HeLa cells. The complexes between the fluorescent primary antibody (3 µl) and PULSin™ (1 µg BSA 1 µl Ab 2 µl Ab) were generated according to the standard protocol and added to HeLa cells. Pictures were taken after 16 hours.

Figure 8: Efficiency of intracellular R-PE delivery in different cell types using PULSin™. The cell types and corresponding intracellular protein delivery efficiencies are indicated: adherent cell lines, adherent primary cells and non-adherent cell lines are shown. Pictures show R-PE delivery in specific cell types.

**References**


**Conclusion**

Delivery of proteins to live cells presents a wide range of applications. As demonstrated in this article, PULSin™ is a potent reagent for protein delivery. PULSin™ promotes efficient crossing of the plasma membrane, protein release from the endosome and allows protein diffusion within the cytoplasm. PULSin™ is a versatile reagent that is able to form complexes with various proteins and deliver them to a wide variety of cells. Indeed, only few parameters need optimizing to obtain the best results: the amount of protein, the volume of the use solution provided with a fast and simple analysis. For the user, PULSin™ is a ready-to-use solution provided with a fast and simple protocol.

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat. N°</th>
<th>Size</th>
<th>Reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>PULSin™</td>
<td>501-01*</td>
<td>0.1 ml</td>
<td>6</td>
</tr>
<tr>
<td>PULSin™</td>
<td>501-04*</td>
<td>0.4 ml</td>
<td>24</td>
</tr>
<tr>
<td>PULSin™</td>
<td>501-16**</td>
<td>4 x 0.4 ml</td>
<td>96</td>
</tr>
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</table>

* This kit contains 20 µg of R-Phycocerythrin (positive control) and 20 ml of Hapes dilution buffer.
** This kit contains 20 µg of R-Phycocerythrin (positive control) and 4 x 20 ml of Hapes dilution buffer.

For additional information, please contact our technical support service: support@polyplus-transfection.com
Guidelines to set up your gene delivery experiments in mice

General considerations

High-quality plasmid preparation is recommended, RNA and endotoxin-free with a low protein content (OD260/280 ratio should be greater than 1.8).

A sterile isotonic 10% glucose (W/V) solution is recommended to prepare in vivo-jetPEI™/DNA complexes (final concentration 5% glucose), since formation of small and stable in vivo-jetPEI™/DNA complexes is only possible in the absence or at low concentration of salts. The volume of in vivo-jetPEI™ is determined by the N/P ratio which is a measure of the ionic balance of the complexes, referring to the number of nitrogen residues of in vivo-jetPEI™ per DNA phosphate.

All animal experiments must be approved by the local ethics committee and carried out according to the guidelines of the animal life protection law. Mice are maintained on specific rodent food and water at 24±1°C, 55±10% humidity and 12h:12h light-dark cycle under specific pathogen-free conditions. Animals are acclimated for at least 7 days before starting experiments. When needed, mice are anaesthetized by inhalation using anaesthetic methoxyflurane or by intraperitoneal injection of pentobarbital or Ketamine/xylasine.

Tail vein injection

| DNA:         | 50 µg      |
| in vivo-jetPEI™: | 5-10 µl   |
| N/P ratio:   | 5-10      |
| Injection volume: | 200-400 µl, 5% glucose |

Method: The mouse is placed in a restrainer and 70% ethanol is applied on the tail in order to slightly swell the vein. Complexes in solution are injected into the tail vein over 10 sec, using a ½ inch 26 gauge needle and a 1 ml syringe.

Intraperitoneal injection

| DNA:         | 100 µg     |
| in vivo-jetPEI™: | 16-20 µl  |
| N/P ratio:   | 8-10      |
| Injection volume: | 400 µl to 1 ml, 5% glucose |

Method: Complexes in solution are injected into the peritoneal cavity over 10 sec, using a ½ inch 26 gauge needle and a 1 ml syringe.
**Intracerebral injection (stereotaxic injection)**

1 µg (for 8-12 week-old mice)

**in vivo-jetPEI™:** 0.12 µl

**N/P ratio:** 6

**Injection volume:** 5 µl, 5% glucose

**Method:** Single injection (5 µl) into either ventricle (0.2 mm posterior to the sylvian fissure, 0.1 mm lateral, and 0.2 mm from the pial surface) to pentobarbital anesthetized mice (65 mg/kg).

![Image of transfected cells expressing the luciferase and found in the anterior lateral ventricle (1 week after intraventricular of pCMVLacZ). Demeneix](image)

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**Retro-orbital injection**

DNA: 40 µg

**in vivo-jetPEI™:** 6.4 µl

**N/P ratio:** 8

**Injection volume:** 200-400 µl, 5% glucose

**Method:** Inserting the tip of a 27g hypodermic needle is introduced carefully in front of the eye. Follow the edge of the orbit down until feeling the needle tip at the base beneath the eye. Inject complexes in solution within 2 sec. If performed carefully, there will be little or no bleeding. The capillary nexus will take up the injected solution rapidly.

![Tissue distribution of luciferase transgene expression 24 h following retro-orbital injection.](image)

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**Nasal instillation for trachea and lung delivery**

DNA: 20 µg

**in vivo-jetPEI™:** 2.4 µl

**N/P ratio:** 5-10

**Injection volume:** 50-200 µl, 5% glucose

**Method:** Mice are held supine at an angle of 45° with pressure applied to the lower mandible to immobilize the tongue and prevent swallowing. Complexes in solution are then introduced to the nasal planum using a micropipet.

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**Intratumoral injection**

DNA: 10-20 µg

**in vivo-jetPEI™:** 2.4 µl

**N/P ratio:** 5-10

**Injection volume:** 50-100 µl, 5% glucose

**Method:** For implanted subcutaneous tumors (size> 5 mm³), perform multiple injections of 10-20 µl complexes at different sites of the tumor to avoid reflux.

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**Nebulization for trachea and lung delivery**

DNA: 40 µg

**in vivo-jetPEI™:** 4.8 µl

**N/P ratio:** 5-10

**Nebulized volume:** 4-10 ml, 5% glucose

**Method:** Mice are placed unrestrained in a whole-body aerosol exposure device. Complexes are aerosolized using an air-jet nebulizer operated with a frequency of approximately 2 MHz and 10 L/min air flow rate. 4-10 ml of solution are nebulized for 15-30 min.

For information regarding other delivery routes than those mentioned here or other animal models, please contact our Technical Support that will be pleased to assist you: support@polyplus-transfection.com

www.polyplus-transfection.com
INTERFERin™ is a new-generation reagent for siRNA transfection that achieves over 90% silencing efficiency with 1 nM of siRNA in a wide variety of adherent and primary cells with excellent cell viability. The use of low siRNA concentrations avoids unwanted toxic and off-target (non-specific) effects thus giving accurate and reliable silencing results. INTERFERin™ is ready-to-use and compatible with serum and antibiotics. Reverse and batch transfection protocols also make INTERFERin™ the ideal solution for HTS applications.

**Less is more: with INTERFERin™, you’ll get more valuable data using less siRNA...**

INTERFERin™-mediated delivery of GL3Luc siRNA to A549Luc cells stably expressing the GL3 luciferase gene shows selective and highly efficient knockdown of gene expression. Amazingly, 50% silencing is still achieved at 10 pM siRNA in the presence of serum (Figure 1). Using subnanomolar siRNA concentrations avoids unwanted toxic and off-target effects associated with reagents requiring higher siRNA concentrations.

INTERFERin™ also reduces lamin A/C gene expression to barely detectable level using 1 nM siRNA (Figure 2).

**Efficient in many cell types: Over 90% gene silencing in a wide variety of cells**

For adherent cell lines or primary cells, 1 nM or lower siRNA concentrations are sufficient to obtain more than 90% gene silencing, regardless of the target gene. For difficult-to-transfect suspension cell lines, 80% silencing can still be reached with INTERFERin™ using 5 nM siRNA (Table 1).

![A549-GL3Luc cells / 24-well plate](image1)

<table>
<thead>
<tr>
<th>siRNA concentration</th>
<th>% inhibition</th>
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<tr>
<td>1 nM</td>
<td>100%</td>
</tr>
<tr>
<td>100 pM</td>
<td>90%</td>
</tr>
<tr>
<td>10 pM</td>
<td>80%</td>
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Figure 1. INTERFERin™-mediated siRNA transfection inhibits luciferase expression in A549-GL3Luc cells. Cells were transfected in 24-well plates in the presence of serum with decreasing concentrations of luciferase siRNA (GL3Luc) duplexes using INTERFERin™. Luciferase expression was measured after 48 h. No inhibition was observed with control siRNA duplexes (mismatch GL2Luc, data not shown).

Table 1. Silencing efficiency in selected cells using INTERFERin™.

<table>
<thead>
<tr>
<th>Adherent cell lines (1 nM siRNA)</th>
<th>% inhibition</th>
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<tbody>
<tr>
<td>A549</td>
<td>&gt; 90%</td>
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<tr>
<td>HeLa</td>
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<tr>
<td>CaSki</td>
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<td>MCF7</td>
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<td>NIH-3T3</td>
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<tr>
<td>SiHa</td>
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<table>
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<tr>
<th>Primary cells (1 nM siRNA)</th>
<th>% inhibition</th>
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<tbody>
<tr>
<td>Primary human fibroblasts</td>
<td>&gt; 90%</td>
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<tr>
<td>Primary human hepatocytes</td>
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<table>
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<tr>
<th>Suspension cell lines (5 nM siRNA)</th>
<th>% inhibition</th>
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<tbody>
<tr>
<td>K562</td>
<td>&gt; 80%</td>
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<td>THP-1</td>
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Superior transfection results... Less toxicity......

INTERFERin™ was compared to three other reagents at nanomolar concentrations. INTERFERin™ was clearly the most efficient at lower concentrations (Figure 3).

In the same experiment (48 h after transfection at 1 nM siRNA), cells transfected with INTERFERin™ or with reagent H appeared healthy, while toxicity was observed with reagent S (Figure 4).

Extremely simple protocol...

INTERFERin™ is ready-to-use and the protocol is straightforward. 1 nM siRNA is recommended as a starting concentration for silencing experiments (Figure 5).

No medium changes are required after transfection. INTERFERin™ can be left on the cells without any adverse effects.

Extremely simple protocol...


Protocols for reverse and batch transfections are available too. They provide the same robust silencing at 1 nM siRNA.
New products

jetPEI™-Hepatocyte, an efficient DNA transfection reagent for Hepatocytes

- Up to 50% efficiency in hepatocyte-like cells and primary hepatocytes
- Gentle to cells
- Easy-to-use protocol
- Compatible with serum and antibiotics

jetPEI™-Hepatocyte is an easy-to-use DNA transfection reagent dedicated to hepatocytes. This ligand-conjugated jetPEI™ enhances transfection of cells expressing galactose-specific membrane lectins, such as hepatocytes. Up to 50% transfection efficiency can be reached for primary human hepatocytes as well as hepatocyte-derived cell lines such as BNL-CL.2

Higher transfection efficiency compared to the versatile jetPEI™

Compared to our standard jetPEI™ transfection reagent, jetPEI™-Hepatocyte provides significantly higher transfection efficiencies on human hepatocarcinoma cells HepG2 as well as on the difficult-to-transfect primary human hepatocytes (Figure 1).

50% transfection efficiency and excellent viability in primary hepatocytes

24 h and 48 h after transfection of the pCMVEGFPluc plasmid, primary human hepatocytes show 50% transfection efficiency. In addition, microscopic examination of the cells shows a healthy population (Figure 2).

Mechanism of transfection using jetPEI™-Hepatocyte

jetPEI™-Hepatocyte is a galactose-bearing linear polyethylenimine designed to enhance the transfection of cells expressing galactose-specific membrane lectins, such as hepatocytes expressing the asialoglycoprotein receptor (ASGPR) or Gal/GalNAc receptor. Cell targeting occurs as a result of specific binding of the galactose residues to the cell-surface receptors, leading to internalization of the DNA complexes.

Easy-to-use protocol

The jetPEI™-Hepatocyte protocol is as simple as that used for jetPEI™: mix the DNA with the reagent to form complexes and simply add the mixture to cells. No media changes or washing steps are required since jetPEI™-Hepatocyte is compatible with serum and antibiotics. Transgenic protein expression is determined 24 h to 72 h post-transfection.

Transfection of hepatocyte-derived cell lines made easy

The most commonly used hepatocyte-derived cells BNL-CL.2 and HepG2 were successfully transfected using jetPEI™-Hepatocyte and usually show approximately 50% to 30% transfection efficiency, respectively (Figures 3 and 4).

Targeting of hepatocyte-like cells using jetPEI™-Hepatocyte

BNL-CL.2 and HepG2 cells were successfully transfected using jetPEI™-Hepatocyte. Transfection of cells expressing galactose-specific membrane lectins, such as hepatocytes, was determined 48h post-transfection. The transfection efficiency was expressed as ng of luciferase per mg of protein.

Higher transfection efficiency compared to the versatile jetPEI™

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Products citation

A new online tool for your literature searches

Under the section Technical support, you will find a powerful tool enabling you to search the citations published by your peers featuring Polyplus-transfection reagents.

Search page

Tick the application you are interested in (in vitro transfection or in vivo delivery), and if you wish, enter the biomolecule, the cell type, the delivery route and/or the keyword of your choice.

Results page

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Abstract page

You will also be able to view the abstract. Downloading of the references is fast and easy: if you click on the reference, you will be automatically redirected to the journal saving you time and effort.

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