INTERFERin®

in vitro siRNA/miRNA transfection reagent

PROTOCOL

DESCRIPTION

INTERFERin® is a powerful siRNA/miRNA transfection reagent that ensures efficient gene silencing and reproducible transfection in mammalian cells. **INTERFERin®** provides more than 90% silencing efficiency at 1 nM siRNA in a wide variety of cells such as HeLa, MCF7or NIH-3T3; hence avoiding off-target effects. For difficult-to-transfect suspension cell lines such as K562 or THP-1 cells, 80% silencing is observed with **INTERFERin®** using a final siRNA concentration of 5 nM. Find relevant publications and transfection conditions for your experiments in the Polyplus-transfection® database on www.polyplus-transfection.com.

1 STANDARD siRNA TRANSFECTION OF ADHERENT CELLS

1.1 CELL SEEDING

For optimal transfection of standard adherent cells using INTERFERin®, cells should be seeded the day before transfection to reach 30-50% confluency at the time of transfection (refer to Table 1 for the recommended number of cells to seed according to the culture vessel formats).

Table 1. Recommended number of cells to seed the day before transfection.

Culture vessel	Number of adherent cells to seed	Surface area per well (cm^2)	Volume of medium per well to seed the cells (ml)
96-well	5 000 ± 2 500	0.3	0.2
24-well	25 000 ± 10 000	1.9	1
12-well	50 000 ± 20 000	3.8	2
6-well / 3.5 cm	150 000 ± 50 000	9.4	4
6 cm / flask 25 cm^2	400 000 ± 100 000	25 - 28	8
10 cm / flask 75 cm^2	1 x 10^6 ± 250 000	75 - 78.5	15
14 cm / flask 175 cm^2	2 x 10^6 - 5 x 10^6	153 - 175	20

1.2 TRANSFECTION OF ADHERENT CELLS

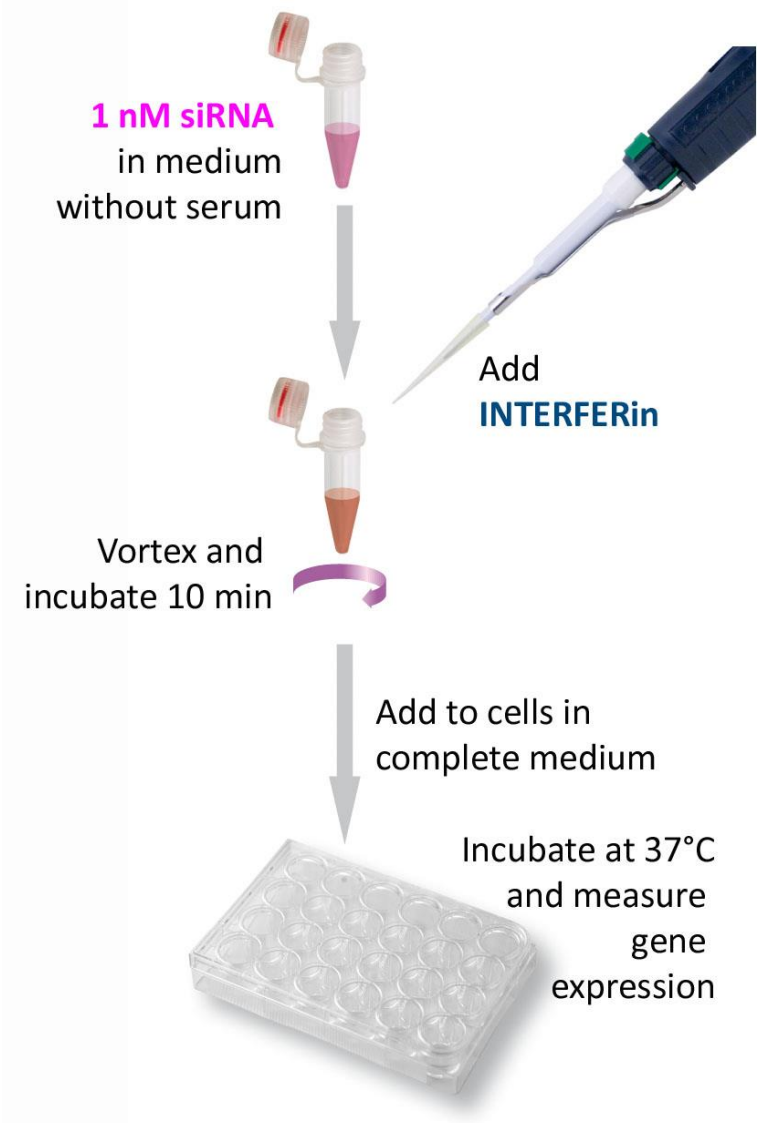
As starting conditions for your gene silencing experiment, we recommend testing siRNA concentrations ranging from 1 nM to 10 nM, as the optimal siRNA concentration depends largely on the target gene, the cell type, the siRNA potency, the half-life of the target mRNA and the turnover of the target protein. Please note that off-target effects are usually minimized at lower siRNA concentrations. The volume of INTERFERin® should be adjusted according to the siRNA concentration and the plate size as shown in Table 2. The transfection conditions are detailed in Table 3 for all culture plate formats.

Recommendations:

- *Check the concentration of the siRNA duplexes, even if provided by the manufacturer.*
- *Use RNAse free and apyrogenic materials such as tips, tubes, buffers.*

1.2.1 siRNA TRANSFECTION PROTOCOL USING 1 nM siRNA

The following protocol is given for transfection of siRNA duplexes at 1 nM per well in a 24-well plate, refer to Table 2 for transfection in other culture formats.



1. For each well, dilute 0.6 pmoles (8.4 ng) of siRNA duplexes into 100 µl of medium without serum or in Opti-MEM®. Mix by pipetting up and down.

2. Vortex INTERFERin® reagent for 5 sec and spin down before use.

3. Add 2 µl of INTERFERin® to the 100 µl of siRNA duplexes.

4. Immediately homogenize by vortexing for 10 seconds.

5. Incubate for 10 minutes at room temperature to allow transfection complexes to form between siRNA duplexes and INTERFERin®. Do not exceed 30 min.

6. During complex formation, remove the growth medium and add 0.5 ml of fresh pre-warmed complete medium per well.

7. Add 100 µl of transfection mix onto the cells and homogenize by gently swirling the plate. The final volume is 600 µl and the siRNA concentration is 1 nM.

8. Incubate the plate at 37°C.

9. Gene silencing is usually measured between 24 to 72 h for mRNA levels and 48 to 96 h for proteins.

Table 2. Recommended transfection conditions in various cell culture formats at 1 nM siRNA

Culture vessel	siRNA duplexes (pmoles)	Amount of siRNA per well	Volume of INTERFERin®	Volume of medium w/o serum for complexation	Volume of complete medium on cells	Final volume
96-well	0.17	2.4 ng	0.75 ± 0.5 µl	50 µl	125 µl	175 µl
24-well	0.6	8.4 ng	2 ± 1 µl	100 µl	500 µl	600 µl
12-well	1.2	17 ng	4 ± 2 µl	200 µl	1 ml	1.2 ml
6-well / 3.5 cm	2.2	31 ng	8 ± 4 µl	200 µl	2 ml	2.2 ml
6 cm / flask 25 cm^2	4.4	62 ng	15 ± 5 µl	400 µl	4 ml	4.4 ml
10 cm / flask 75 cm^2	10.5	147 ng	40 ± 10 µl	500 µl	10 ml	10.5 ml

1.2.2 TRANSFECTION CONDITIONS USING 10 TO 50 nM siRNA

When working at siRNA concentrations ranging from 10 to 50 nM, use recommended conditions indicated in Table 3.

Table 3. Recommended conditions to transfect adherent cells in different cell culture vessels from 10 to 50 nM siRNA

Culture vessel	Volume of INTERFERin®	Volume of medium w/o serum for complexe formation	Volume of complete medium on cells	Final volume
96-well	1 ± 0.5 µl	50 µl	125 µl	175 µl
24-well	3 ± 1 µl	100 µl	500 µl	600 µl
12-well	6 ± 2 µl	200 µl	1 ml	1.2 ml
6-well / 3.5 cm	12 ± 4 µl	200 µl	2 ml	2.2 ml
6 cm / flask 25 cm^2	20 ± 5 µl	400 µl	4 ml	4.4 ml

2 siRNA TRANSFECTION OF SUSPENSION CELLS

2.1 CELL SEEDING

For optimal transfection conditions of suspension cells with INTERFERin®, cells should be seeded the day of transfection in a reduced volume compared to usual culture conditions. Refer to Table 4 for the recommended number of cells to seed according to the culture vessel formats and for the advised volume of complete medium.

Table 4. Recommended number of suspension cells to seed the day of transfection

Culture vessel	Number of suspension cells to seed the day of transfection	Volume of medium per well
384-well	5 000 - 10 000	25 µl
96-well	10 000 - 20 000	50 µl
24-well	100 000 - 200 000	200 µl
12-well	200 000 - 400 000	500 µl
6-well / 3.5 cm	500 000 - 2 x 10^6	1 ml
6 cm / flask 25 cm^2	2 x 10^6 - 5 x 10^6	2 ml

2.2 siRNA TRANSFECTION OF SUSPENSION CELLS

In order to optimize endogenous gene silencing, we recommend testing a range of siRNA concentrations from 5 nM to 20 nM. The volume of INTERFERin® needs to be adjusted accordingly, depending on the siRNA concentration as described in Table 5. For detailed transfection conditions at 5 nM siRNA, please refer to Table 6.

Table 5. Recommended volumes of INTERFERin® according to the siRNA concentration and the plate format for transfection of cells grown in suspension.

Final siRNA concentration	Plate format	Volume of INTERFERin® reagent/well
1 to 20 nM	384-well	1 ± 0.5 µl
	96-well	2 ± 1 µl
	24-well	3 ± 2 µl
	6-well or 35 mm	10 ± 8 µl
20 to 50 nM	384-well	1.5 ± 0.5 µl
	96-well	3 ± 1 µl
	24-well	5 ± 2 µl
	6-well or 35 mm	15 ± 8 µl

Preparation of the complexes and transfection procedure

The following protocol is given for transfection of siRNA duplexes at 5 nM per well in a 24-well plate. See Table 6 for transfection in other culture formats.

1. For each well, dilute 1.5 pmoles (21 ng) of siRNA duplexes into 100 µl medium without serum or in Opti-MEM®. Mix by pipetting up and down.
2. Vortex INTERFERin® reagent for 5 sec and spin down before use.
3. Add 4 µl of INTERFERin® to the 100 µl siRNA duplexes solution.
4. Mix immediately for 10 seconds (vortex).
5. Incubate for 15 minutes at room temperature to allow INTERFERin®/siRNA complexes to form (do not exceed 30 min).
6. Add the 100 µl INTERFERin®/siRNA mix per well into 0.2 ml of cells suspension in growth medium, and homogenize by gently swirling the plate. The final volume is 300 µl and the siRNA concentration is 5 nM.
7. Incubate the plate at 37°C.
8. After 4 to 6 hours, add 0.7 ml of complete medium and incubate as before.
9. Gene silencing is usually measured between 24 to 72 h for mRNA levels and 48 to 96 h for proteins.

Table 6. Recommended conditions for siRNA transfection at 5 nM in suspension cells

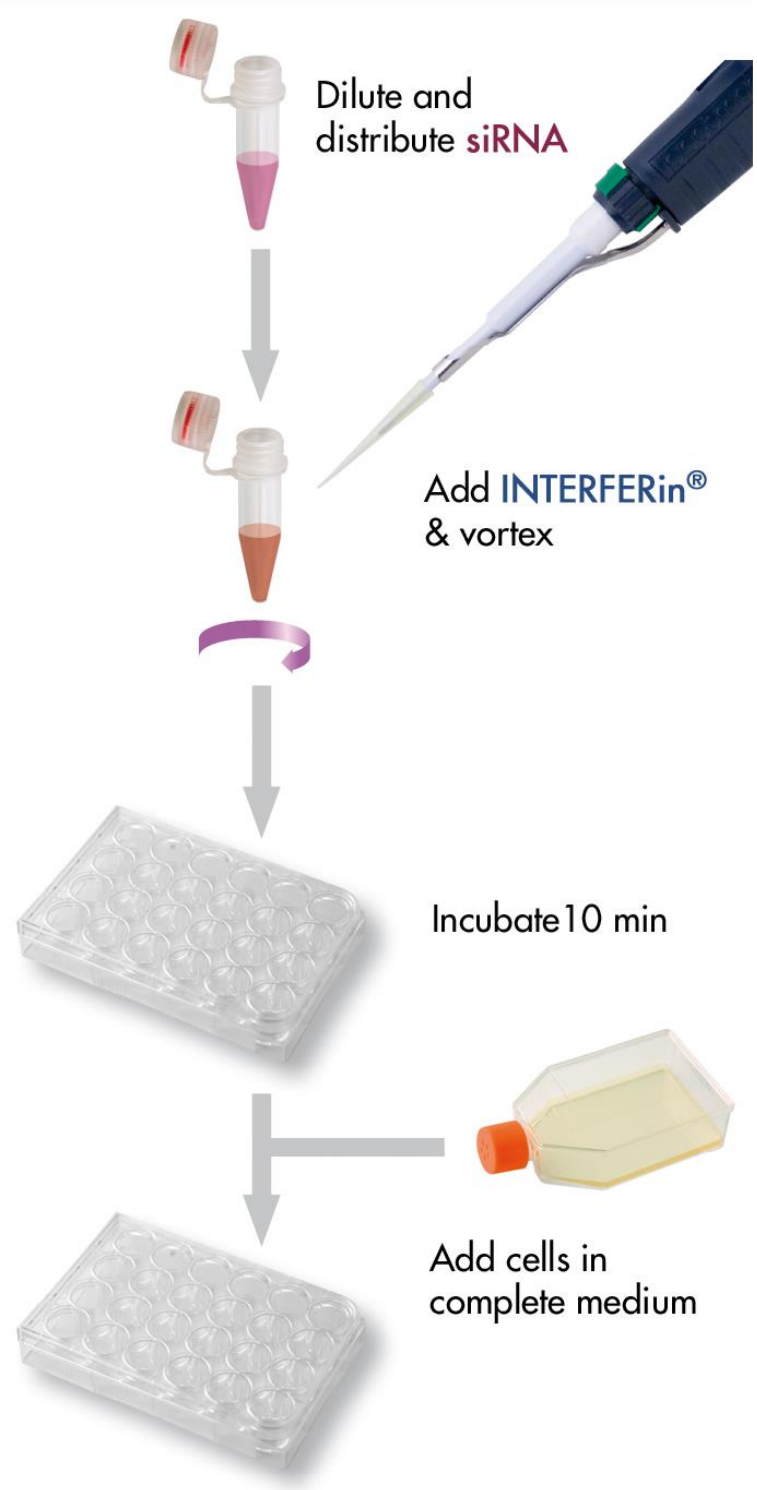
Culture vessel	Volume of cell suspension	siRNA duplexes (pmoles)	Amount of siRNA per well	Volume of INTERFERin®	Volume of medium w/o serum for complexation	Volume of medium to add after 4 - 6 h
384-well	25 µl	0.25	3.75 ng	1 ± 0.5 µl	25 µl	0 µl
96-well	50 µl	0.5	7.5 ng	2 ± 1 µl	50 µl	100 µl
24-well	200 µl	1.5	21 ng	3 ± 2 µl	100 µl	0.7 ml
12-well	500 µl	3.5	49 ng	6 ± 4 µl	200 µl	1 ml
6-well / 3.5 cm	1 ml	6	84 ng	10 ± 8 µl	200 µl	2 ml
6 cm / flask 25 cm^2	2 ml	12	168 ng	15 ± 10 µl	400 µl	4 ml

Recommendations:

- *For other siRNA concentrations, please adjust the conditions accordingly.*

3 REVERSE TRANSFECTION PROTOCOL FOR HTS

In this procedure, siRNA and INTERFERin® reagent are added or prepared in the wells and the cells are overlayed subsequently (see figure below). This optimized protocol is a time saving protocol, in which transfection and plating are performed on the same day. This procedure is suitable for automated experiments and particularly for High Throughput Screening (HTS) applications.



3.1 PREPARATION OF THE CELLS

Trypsinize the cells and prepare a cell suspension in growth medium at the recommended cell density according to Table 7.

Table 7. Recommended number of cells for different cell culture vessels.

Culture vessel	Number of cells added per well	Volume of cells per well	Minimal volume of cell suspension per plate (cells/ml)	Number of cells to prepare per plate
384-well	2 500 ± 500	50 µl	20 ml (50 000 cells/ml)	1 000 000 ± 200 000
96-well	7 500 ± 2 500	125 µl	12.5 ml (60 000 cells/ml)	750 000 ± 250 000
24-well	40 000 ± 10 000	500 µl	12.5 ml (100 000 cells/ml)	1 250 000 ± 250 000

3.2 OPTIMIZING siRNA CONCENTRATION

Using reverse transfection, INTERFERin® enables efficient silencing (> 90 %) of many genes with 1 nM siRNA in the presence of serum. However, the optimal siRNA concentration depends largely on the target gene, the cell type, the siRNA potency, the half-life of the target mRNA and the turnover of the target protein. Thus, we recommend optimizing your gene silencing experiment. As a starting condition, we suggest testing siRNA concentrations ranging from 1 nM to 20 nM. Please note that off-target effects are usually minimized at lower siRNA concentrations. The transfection conditions for each cell culture plate format are described in Table 8.

Table 8. Recommended conditions for siRNA transfection at 1 nM in various cell culture vessels.

Culture vessel	siRNA duplexes (pmoles)	Amount of siRNA per well	Volume of medium w/o serum for complexation	Volume of INTERFERin® per well	Volume of cells in complete medium	Final total volume
384-well	0.06	0.84 ng	15 µl	0.5 ± 0.25 µl	45 µl	60 µl
96-well	0.17	2.4 ng	50 µl	0.75 ± 0.5 µl	125 µl	175 µl
24-well	0.6	8.4 ng	100 µl	2 ± 1 µl	500 µl	600 µl

In order to improve pipetting accuracy when dispensing small volumes of INTERFERin®, you may dilute INTERFERin® 5-fold in water and add 5 volumes of diluted INTERFERin® solution per well. When working at siRNA concentrations from 10 to 50 nM, use the conditions indicated in Table 9.

Table 9. Recommended conditions for transfection from 10 to 50 nM siRNA in various cell culture vessels.

Culture vessel	Volume of medium w/o serum for complexation	Volume of INTERFERin® per well	Volume of cells in complete medium	Final total volume
384-well	15 µl	0.5 ± 0.25 µl	45 µl	60 µl
96-well	50 µl	0.75 ± 0.5 µl	125 µl	175 µl
24-well	100 µl	2 ± 1 µl	500 µl	600 µl

3.3 REVERSE TRANSFECTION PROTOCOL

The following protocol is given for transfection of siRNA duplexes at 1 nM per well in a 96-well plate. These conditions are provided as starting point for optimization of siRNA transfection. Refer to Table 8 for transfection in other culture formats.

1. For each well, dilute 0.17 pmoles (2.4 ng) of siRNA duplexes into 50 µl of medium without serum or in Opti-MEM®.
2. Lay 50 µl of pre-homogenized siRNA solution onto the well (or prepare a mastermix in a tube).
3. Vortex INTERFERin® reagent for 5 sec and spin down before use.
4. Add 1 µl of INTERFERin® to the 50 µl of siRNA solution.
5. Mix promptly by agitating the plate on an orbital shaker for 5 min, or pipetting up and down.
6. Incubate for 10 minutes at room temperature to allow transfection complexes to form (do not exceed 30 min).
7. Add 7500 cells per well (125 µl at 60 cells/µl) in complete culture medium onto the siRNA/INTERFERin® complexes solution. The final volume per well is 175 µl and the siRNA concentration is 1 nM. Mix gently by moving the plate in a figure of 8.
8. Incubate the plate at 37°C.
9. Gene silencing is usually measured between 24 to 72 h for mRNA levels and 48 to 96 h for proteins.

3.4 REVERSE TRANSFECTION FOR AUTOMATED PROCEDURE

The protocol is given for automated transfection of siRNA duplexes at 1 to 20 nM per well. Prior to use, **dilute INTERFERin® 5-fold in water**. Refer to Table 10 for starting conditions for siRNA transfection.

Table 10. Recommended transfection conditions for automated approaches.

Culture vessel	Volume of resuspended siRNA per well	Volume of diluted INTERFERin® per well	Volume of diluted INTERFERin® per plate	Volume of cell suspension per well	Minimal volume of cell suspension required per plate
384-well	15 µl	2.5 µl	1 ml	45 µl (2 500 cells)	20 ml (50 000 cells/ml)
96-well	50 µl	5 µl	0.5 ml	125 µl (7 500 cells)	15 ml (60 000 cells/ml)

When using a robot, take into account the dead volume within the apparatus (usually 3 to 5 ml) and prepare a sufficient volume of each reagent and cells.

The following protocol is given for automated transfection in a 384-well plate.

1. Add 15 µl of siRNA into the well, prepared as recommended by the manufacturer.
2. Vortex INTERFERin® reagent for 5 sec and spin down before use.
3. Add 2.5 µl of the 5-fold diluted solution of INTERFERin® to the siRNA solution and mix by pipetting up and down.
4. Incubate for 10 minutes at room temperature to allow transfection complexes to form (do not exceed 30 min).
5. Add 2500 cells per well in cell growth medium onto the siRNA/INTERFERin® complexes solution. The final volume per well is 60 µl. Mix gently by moving the plate in a figure of 8.
6. Incubate the plate at 37°C.
7. Gene silencing is measured between 24 - 72 h for mRNA levels and 48 – 96 h for proteins.

Recommendations

- *The dispensed volumes of siRNA and of diluted INTERFERin® can be adapted to the robot.*

4 miRNA TRANSFECTION

INTERFERin® is suitable for transfection of miRNA and miRNA-related molecules by using the standard protocol, described in Section 1.2. for adherent and Section 2.2. for suspension cells.

5 TROUBLESHOOTING

Observations	Actions
Low silencing efficiency	Increase the siRNA concentration per well.
	Increase the volume of INTERFERin® per well.
	Check silencing efficiency at various time points after transfection from 24 to 96 h.
	Use Opti-MEM® to dilute the siRNA.
	Ensure that adherent cells are 30-50% confluent the day of transfection. For small cells and slow growing cell types, seed approximately 2 times more cells per well to reach the adequate confluence.
	Check all reagents are RNase free.
	Ensure that your siRNAs are high-quality (PAGE purified and desalted).
	Check siRNA duplexes concentration and annealing.
	Decrease the volume during transfection by half and gently centrifuge the plate (5 min at 180 g). After 4 hours, add medium to restore the usual culture volume
	For reverse transfection, INTERFERin/siRNA complexes prepared in medium without serum or in Opti-MEM® should be used within the following 2 hours.
Cellular toxicity	Reduce the incubation time of INTERFERin®/siRNA complexes with the cells by changing medium 4 to 6 h after transfection or simply adding medium to the well.
	Decrease the volume of INTERFERin® used in the transfection assay.
	Check that silencing the target gene does not affect cell viability.

6 PRODUCT INFORMATION

6.1 ORDERING INFORMATION

Ref #	Volume of INTERFERin® siRNA/miRNA Transfection reagent
409-01	0.1 ml
409-10	1 ml
409-50	5 x 1 ml

6.2 CONTENT

One ml of INTERFERin® transfection reagent is sufficient to perform ca. 500 to 1000 transfections (using 1 nM of siRNA) in 24-well plates.

6.3 REAGENT USE AND LIMITATIONS

For research use only. Not for use in humans.

6.4 QUALITY CONTROL

Every batch of INTERFERin® is tested in house in a transfection assay on A549-Luc cells, constitutively expressing the Luciferase gene. The silencing efficiency obtained using 1 nM siRNA and INTERFERin® for each batch is indicated on the Certificate of Analysis.

6.5 FORMULATION AND STORAGE

INTERFERin® should be stored tightly capped at 4°C upon arrival. **Do not freeze.** INTERFERin®, as guaranteed by the Certificate of Analysis, will be stable for at least 6 months (409-01) to at least one year (other packaging sizes) when stored appropriately. Polyplus-transfection® has been awarded ISO 9001 Quality Management System Certification since 2002, which ensures that the company has established reliable and effective processes for manufacturing, quality control, distribution and customer support.

6.6 TRADEMARKS

Polyplus-transfection and INTERFERin are registered trademarks of Polyplus-transfection. OptiMEM® is registered trademark of Life Technologies Corporation. How to cite us: “INTERFERin® (Polyplus-transfection S.A, Illkirch, France)”

6.7 TECHNICAL ASSISTANCE AND SCIENTIFIC ADVICE

Contact the friendly Polyplus technical support *via*:

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- Email: support@polyplus-transfection.com
- Phone: +33 3 90 40 61 87