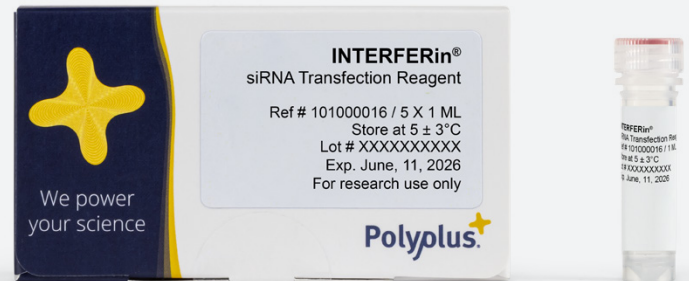


INTERFERin®

In Vitro siRNA | miRNA
Transfection Reagent



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, MCF7 or 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 on www.sartorius.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	0.125
24-well	25,000 ± 10,000	1.9	0.5
12-well	50,000 ± 20,000	3.8	1
6-well/35 mm	150,000 ± 50,000	9.4	2
60 mm/flask 25 cm ²	400,000 ± 100,000	25–28	5
100 mm/flask 75 cm ²	1 × 10 ⁶ ± 250,000	75–78.5	10
140 mm/flask 175 cm ²	2 × 10 ⁶ –5 × 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.

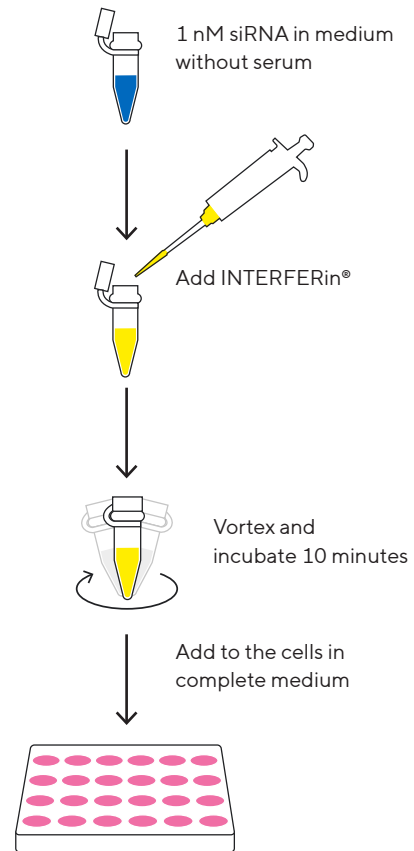
Recommendations:

- 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 seconds 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 minutes.
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 hours for proteins.



Incubate at 37 °C and measure gene expression

Table 2: Recommended Transfection Conditions in Various Cell Culture Formats at 1 nM siRNA

Culture vessel	siRNA duplexes [pmoles]	Amount of siRNA per well [ng]	Volume of INTERFERin® reagent [μ L]	Volume of medium w/o serum for complexation [μ L]	Volume of complete medium on cells	Final volume
96-well	0.17	2.4	0.75 \pm 0.5	50	125 μ L	175 μ L
24-well	0.6	8.4	2 \pm 1	100	500 μ L	600 μ L
12-well	1.2	17	4 \pm 2	200	1 mL	1.2 mL
6-well/35 mm	2.2	31	8 \pm 4	200	2 mL	2.2 mL
60 mm/flask 25 cm ²	4.4	62	15 \pm 5	400	4 mL	4.4 mL
100 mm/flask 75 cm ²	10.5	147	40 \pm 10	500	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® reagent [μ L]	Volume of medium w/o serum for complexation [μ L]	Volume of complete medium on cells	Volume of medium w/o serum for complexation
96-well	1 \pm 0.5	50	125 μ L	175 μ L
24-well	3 \pm 1	100	500 μ L	600 μ L
12-well	6 \pm 2	200	1 mL	1.2 mL
6-well/35 mm	12 \pm 4	200	2 mL	2.2 mL
60 mm/flask 25 cm ²	20 \pm 5	400	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 μ L
96-well	10,000 - 20,000	50 μ L
24-well	100,000 - 200,000	200 μ L
12-well	200,000 - 400,000	500 μ L
6-well/35 mm	500,000 - 2 x 10 ⁶	1 mL
60 mm/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 INTERFERin® reagent/well [µL]
1 to 20 nM	384-well	1±0.5
	96-well	2±1
	24-well	3±2
	6-well or 35 mm	10±8
20 to 50 nM	384-well	1.5±0.5
	96-well	3±1
	24-well	5±2
	6-well or 35 mm	15±8

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 seconds 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 minutes).
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 hours for mRNA levels and 48 to 96 hours for proteins.

Table 6: Recommended Conditions for siRNA Transfection at 5 nM in Suspension Cells

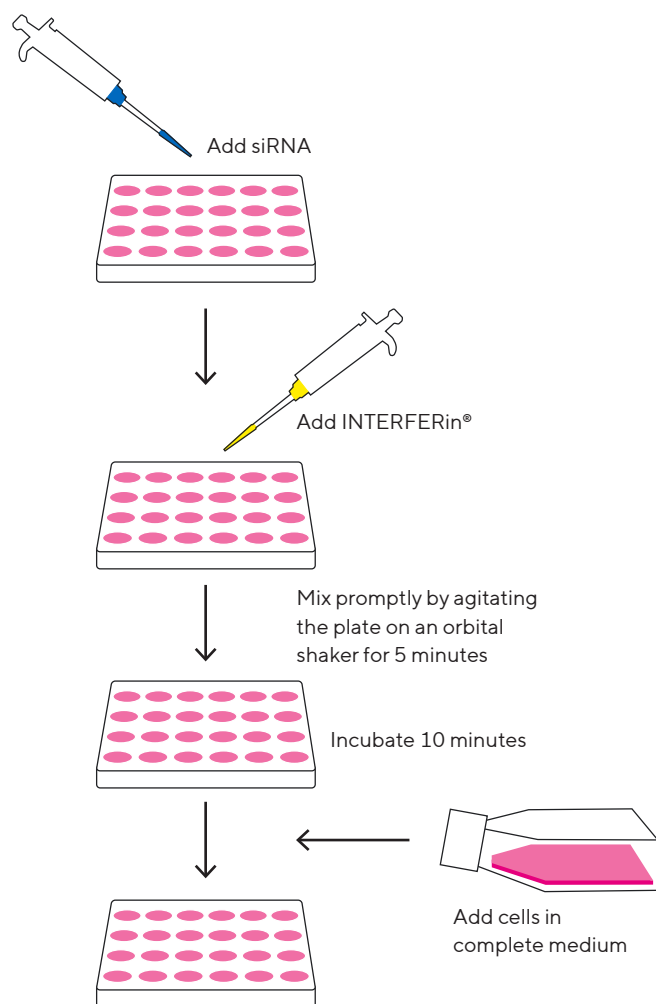
Culture vessel	Volume of cell suspension	siRNA duplexes [pmoles]	Amount of siRNA per well [ng]	Volume of INTERFERin® reagent [µL]	Volume of medium w/o serum for complexation [µL]	Volume of medium to add after 4–6 hours
384-well	25 µL	0.25	3.75	1±0.5	25	0 µL
96-well	50 µL	0.5	7.5	2±1	50	100 µL
24-well	200 µL	1.5	21	3±2	100	0.7 mL
12-well	500 µL	3.5	49	6±4	200	1 mL
6-well/35 mm	1 mL	6	84	10±8	200	2 mL
60 mm/flask 25 cm ²	2 mL	12	168	15±10	400	4 mL

Recommendation:

- 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 overlaid 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 added per well [μ L]	Minimal volume of cell suspension per plate [mL]	Number of cells to prepare per well
384-well	2,500 \pm 500	50	20 (50,000 cells/mL)	1,000,000 \pm 200,000
96-well	7,500 \pm 2,500	125	12.5 (60,000 cells/mL)	750,000 \pm 250,000
24-well	40,000 \pm 10,000	500	12.5 (100,000 cells/mL)	1,250,000 \pm 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 [ng]	Volume of medium w/o serum for complexation [μL]	Volume of INTERFERin® per well [μL]	Volume of in complete medium [μL]	Final volume [μL]
384-well	0.06	0.84	15	0.5±0.25	45	60
96-well	0.17	2.4	50	0.75±0.5	125	175
24-well	0.6	8.4	100	2±1	500	600

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 [μL]	Volume of INTERFERin® per well [μL]	Volume of cells in complete medium [μL]	Final total volume [μL]
384-well	15	0.5±0.25	45	60
96-well	50	0.75±0.5	125	175
24-well	100	2±1	500	600

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 master mix in a tube).
3. Vortex INTERFERin® reagent for 5 seconds 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 10 seconds or pipetting up and down.
6. **Incubate for 10 minutes** at room temperature to allow transfection complexes to form (do not exceed 30 minutes).
7. Add 7,500 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 hours for mRNA levels and 48 to 96 hours for proteins.

3.4 Reverse Transfection per 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 [μL]	Volume of diluted INTERFERin® per well [μL]	Volume of diluted INTERFERin® per plate [mL]	Volume of cell suspension per well [μL]	Minimal volume of cell suspension required per plate [mL]
384-well	15	2.5	1	45 (2,500 cells)	20 (50,000 cells/mL)
96-well	50	5	0.5	125 (7,500 cells)	15 (60,000 cells/mL)

When using a robot, consider 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 seconds 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 minutes).
5. Add 2,500 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 to 72 hours for mRNA levels and 48 to 96 hours for proteins.

Recommendation:

- 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	<ul style="list-style-type: none">▪ 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 hours.▪ 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 minutes at 180 g). After 4 hours, add medium to restore the usual culture volume
Reverse transfection	<ul style="list-style-type: none">▪ INTERFERin®/siRNA complexes prepared in medium without serum or in Opti-MEM™ should be used within the following 2 hours.
Cellular toxicity	<ul style="list-style-type: none">▪ Reduce the incubation time of INTERFERin®/siRNA complexes with the cells by changing medium 4 to 6 hours 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

Part number	INTERFERin® reagent vial size
101000036	0.1 mL
101000028	1 mL
101000016	5x1 mL

6.2 Content

One mL of INTERFERin® transfection reagent is sufficient to perform ca. 500 to 1,000 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.

Certificates of Analysis are available online in the MySartorius portal on www.sartorius.com.

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 (Part N°101000036) to at least one year (other packaging sizes) when stored appropriately.

6.6 Trademarks

Polyplus-transfection® and INTERFIN® are registered trademarks of POLYPLUS-TRANSFECTION S.A. Opti-MEM™ is trademark of Life Technologies Corporation.

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