

# INTERFERin™-HTS

## HTS siRNA Transfection Protocol

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## Company Information

### Technical Assistance and Scientific Advice

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## Product Information

**INTERFERin™-HTS** is a new generation siRNA transfection reagent especially developed for high throughput screening (HTS) applications providing great silencing efficiency and high cell viability with low amounts of reagent.

### Ordering information

Cat #	INTERFERin™-HTS Reagent	Number of transfections
410-002	0.2 ml	25 to 40 transfected 96-well plates
410-015	1.5 ml	200 to 300 transfected 96-well plates
410-060	4 x 1.5 ml	800 to 1200 transfected 96-well plates

### Content

One vial of 1.5 ml of INTERFERin™-HTS transfection reagent is sufficient to transfect *c.a.* 200 to 300 96-well plates.

### Reagent use and Limitations

For research use only. Not for use in humans.

### Quality control

Every batch of INTERFERin™-HTS is tested in house in a reverse transfection assay on A549-Luc cells, stably expressing the luciferase gene. The silencing efficiency obtained using 10 nM siRNA and INTERFERin™-HTS for each batch is indicated on the Certificate of Analysis.

### Formulation and Storage

INTERFERin™-HTS should be stored tightly capped at 4°C upon arrival.

Do not freeze.

INTERFERin™-HTS will be stable for at least six months for cat# 410-002, and at least one year for the other references (cat# 410-015 and 410-060) as indicated on the Certificate of Analysis when stored appropriately.

### Recommendations for siRNA transfection

Check the concentration of the siRNA duplexes, even if provided by the manufacturer. Use RNase free and apyrogenic material such as tips, tubes and buffers.

## 1. Reverse Transfection Protocol

In this procedure, the complexes between siRNA and INTERFERin™-HTS reagent are prepared in the well and the cells are overlaid subsequently. This protocol is suited for automated transfection, as well as for siRNA libraries.

### 1.1 Preparation of the cells

Trypsinize the cells and prepare a cell suspension in growth medium containing serum and antibiotics, at the recommended cell density according to Table 1. Please note that INTERFERin™-HTS is compatible with both serum and antibiotics.

**Table 1. Recommended number of cells to prepare for 96- and 384-well plates.**

Culture vessel	Number of cells to prepare <u>per well</u>	Volume of cells <u>per well</u>	Minimal volume of cell suspension <u>per plate</u>	Number of cells to prepare <u>per plate</u>
96-well	5 000 – 10 000	125 µl	12.5 ml (40 000 – 80 000 cells/ml)	500 000 – 1 000 000
384-well	1 000 – 2 000	50 µl	20 ml (20 000 - 40 000 cells/ml)	400 000 – 800 000

If you wish to transfect less cells, for an apoptotic assay for example, specific protocols are available. Please contact our technical support at [support@polyplus-transfection.com](mailto:support@polyplus-transfection.com).

### 1.2 Reverse transfection protocol

Using reverse transfection, INTERFERin™-HTS enables efficient silencing (> 90 %) of many genes with 10 nM siRNA in the presence of serum and antibiotics. Depending on the target gene, the cell type, the siRNA potency, the half-life of the target mRNA and the turnover of the target protein, we recommend optimizing your gene silencing experiment.

As a starting condition, we suggest testing siRNA concentrations ranging from 10 nM to 50 nM. The transfection conditions for each cell culture plate format are described in Table 2.

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**Dilution of INTERFERin™-HTS:** In order to improve accuracy when dispensing small volumes of reagent, dilute INTERFERin™-HTS in sterile H<sub>2</sub>O so to dispense 5 µl of the diluted solution per well. To do so prepare a master mix (at least for 10 wells *i.e.* 50 µl) and add the recommended volume of INTERFERin™-HTS per well according to your plate size (Table 2).

The diluted INTERFERin™-HTS solution is stable for 4 h at room temperature and for up to one week at 4°C. Please note that INTERFERin™-HTS should not be diluted in OptiMEM®.

The following protocol is given for transfection of siRNA duplexes at 10 nM per well in a 96-well plate. Refer to Table 2 for the protocol in 384-well plates.

1. For each well, dilute 1.80 pmoles siRNA (*c.a.* 24 ng) duplexes into 50 µl of medium without serum, in Opti-MEM® or in 150 mM NaCl\*.
2. Add 50 µl of pre-homogenized siRNA solution to the well.
3. Add 5 µl of diluted INTERFERin™-HTS to the 50 µl of siRNA solution.
4. Incubate for 15-30 minutes at room temperature to allow transfection complexes to form. At this point complexes are stable for 2 h\* at room temperature. Do not store complexes at 4°C.
5. Add 7500 cells per well (125 µl cell suspension at 60 cells/µl) in cell culture medium containing serum and antibiotics to the siRNA/INTERFERin™-HTS complexes. The final volume per well is 180 µl and the siRNA concentration is 10 nM. Mix gently.
6. Incubate the plate at 37°C.
7. Gene silencing is usually measured between 24 to 72 h for mRNA levels and 48 to 96 h for proteins.

\* For siRNA/INTERFERin™-HTS complexes stable for up to 4 h at room temperature, dilute your siRNA in 150 mM NaCl (Polyplus cat# 702-50 & 702-250).

These conditions are provided as starting point. Adjust the siRNA concentration according to your experiment, and if needed the amount of INTERFERin™-HTS but keep the volume of diluted INTERFERin™-HTS solution per well constant (5 µl).

If you wish to perform transfection in 100 µl final volume, specific protocols are available. Please contact our technical support at [support@polyplus-transfection.com](mailto:support@polyplus-transfection.com)

**Table 2. Recommended conditions for siRNA transfection (10 - 50 nM) in 96- and 384-well plates**

Culture vessel	Volume of siRNA per well	Volume of INTERFERin™-HTS needed per well to dilute in water	Volume of <u>diluted</u> INTERFERin™-HTS added per well	Volume of cells in complete medium	Final volume per well
96-well	50 µl	0.05 to 0.075 µl	5 µl	125 µl	180 µl
384-well	15 µl	0.025 to 0.05 µl	5 µl	50 µl	70 µl

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

For cells especially difficult to transfect, the volume of undiluted INTERFERin™-HTS per well of a 96 well plate can be increased to 0.1 µl.

## 2. Forward Transfection Protocol

### 2.1 Cell seeding

For optimal transfection of standard adherent cells using INTERFERin™-HTS, cells should be seeded in complete growth medium the day before transfection to reach 50-70% confluency at the time of transfection (refer to Table 3 for the recommended number of cells to seed according to the culture vessel format). INTERFERin™-HTS transfection reagent is compatible with both serum and antibiotics.

**Table 3. Recommended number of cells to seed the day before transfection**

Culture vessel	Number of adherent cells to seed	Surface area per well	Volume of medium per well to seed the cells
96-well	2500 - 5000	0.3 cm <sup>2</sup>	50 µl
384-well	1000 - 2000	1.6 mm <sup>2</sup>	20 µl

## 2.2 Forward transfection of adherent cells

As starting conditions, we recommend testing siRNA concentrations ranging from 10 nM to 50 nM according to the conditions detailed in Table 4.

The following protocol is given for transfection of siRNA duplexes at 10 nM per well in a 96-well plate, please refer to Table 4 for transfection in 384-well plates.

**For optimal complexes formation, prepare a master mix of siRNA/INTERFERin™-HTS complexes for at least 10 wells.**

1. For each well, dilute 1 pmole (*c.a.* 14 ng) of siRNA duplexes into 50 µl of medium without serum or in Opti-MEM®. Mix by pipetting up and down.
2. Add 0.15 µl of INTERFERin™-HTS to the 50 µl of siRNA duplexes.
3. Immediately homogenize by vortexing for 10 seconds.
4. Incubate 15-30 minutes at room temperature. Do not exceed 2 h.
5. Add 50 µl of transfection mix onto the cells and homogenize by gently swirling the plate. The final volume is 100 µl and the siRNA concentration is 10 nM.
6. Incubate the plate at 37°C.
7. Gene silencing is usually measured between 24 to 72 h for mRNA levels and 48 to 96 h for proteins.

**Table 4. Recommended transfection conditions in 96- and 384-well plates at 10-50 nM siRNA**

Culture vessel	siRNA duplexes (pmoles)	siRNA duplexes concentration	Volume of diluted siRNA in medium per well	Volume of INTERFERin™-HTS	Volume of complete medium on cells	Final volume per well
96-well	1 – 5	10 - 50 nM	50 µl	0.1 to 0.2 µl	50 µl	100 µl
384-well	0.35 – 1.75	10 - 50 nM	15 µl	0.05 to 0.1 µl	20 µl	35 µl

## Troubleshooting

Observations	Actions
Low silencing efficiency	Check all reagents are RNase free.
	Ensure that your siRNA are high-quality (HPLC or PAGE purified and desalted).
	Check siRNA duplexes concentration and annealing.
	Ensure that INTERFERin™-HTS was diluted in ddH <sub>2</sub> O, and not in Opti-MEM®.
	If the siRNA are diluted in Opti-MEM®, use the INTERFERin™-HTS/siRNA complexes in the next 2 hours. If the siRNA are diluted in NaCl, use the INTERFERin™-HTS/siRNA complexes in the next 4 hours.
	Increase the siRNA concentration per well.
	Increase the volume of undiluted INTERFERin™-HTS per well (when using the reverse protocol, keep the volume of diluted INTERFERin™-HTS to 5 µl / well in 96 well plate).
Cellular toxicity	Decrease the volume of INTERFERin™-HTS used in the transfection assay.
	Verify that silencing of the target gene expression is not affecting cell viability.
	Reduce the incubation time of INTERFERin™-HTS /siRNA complexes with the cells by changing medium 4 to 6 h after transfection.