INTERFERin® transfection reagent
Short protocol - siRNA Transfection

DAY 0: Cell seeding

→ Seed cells in V mL of serum containing medium according to the table below

Quantities per well, dish or flask

Culture vessel	Number of cells*	V = volume of medium during transfection
96-well	2 500 – 7 500	0.2 mL
24-well	15 000 – 35 000	1 mL
12-well	30 000 – 70 000	2 mL
6-well / 35 mm	100 000 – 200 000	4 mL
100 mm / flask 75 cm^2	750 000 – 1.25 x 10^6	15 mL

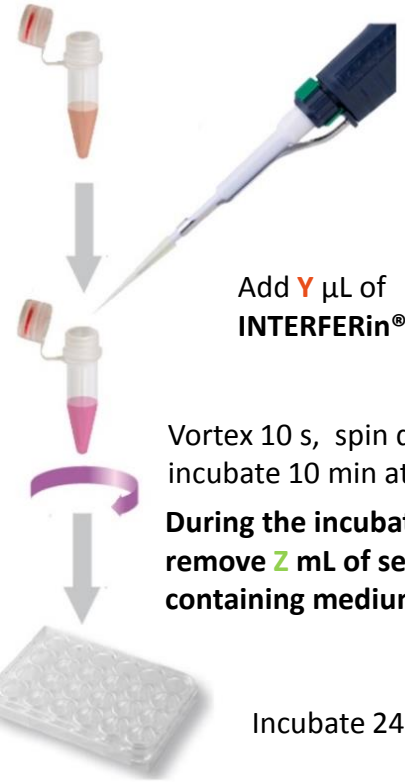
*For suspension cells, please refer to the complete protocol.

DAY 1: Transfection = 1 nM siRNA

→ Perform transfection **in the presence of serum**

→ Transfect cells at **30-50% confluency**

Dilute X pmoles of siRNA in W µL of medium without serum Vortex 10 s and spin down



Add Y µL of **INTERFERin® reagent**

Vortex 10 s, spin down and incubate 10 min at RT

During the incubation time, remove Z mL of serum containing medium

Add transfection mix to the cells in serum containing medium

Incubate 24 to 72 h

Watch the video «siRNA transfection using INTERFERin®» on YouTube!
http://www.youtube.com/watch?v=Yk8W8Cn0zjw

Quantities per well, dish or flask

Culture vessel	W = volume of medium without serum	X = amount of siRNA added (1 nM)	Y = volume of INTERFERin® reagent	Z = volume of serum containing medium
96-well	50 µL	0.17 pmoles (2.4 ng)	0.75 ± 0.5 µL	0.125 mL
24-well	100 µL	0.6 pmoles (8.4 ng)	2 ± 1 µL	0.5 mL
12-well	200 µL	1.2 pmoles (17 ng)	4 ± 2 µL	1 mL
6-well / 35 mm	200 µL	2.2 pmoles (31 ng)	8 ± 4 µL	2 mL
100 mm / flask 75 cm^2	500 µL	10.5 pmoles (147 ng)	40 ± 10 µL	10 mL

DAY 2-3: Analyze gene silencing

See back page for optimization tips

Download complete protocol on http://www.polyplus-transfection.com/resources/product-literature/

Polyplus transfection® THE DELIVERY EXPERTS

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INTERFERin® transfection reagent

Short protocol - Optimization Tips

Protocol Optimization

- The siRNA final concentration may range from 1 to 50 nM depending on the cells and the target gene.
- Cell confluency: between 30 and 50% at the time of transfection.
- Check our online Cell Transfection Database at:

http://www.polyplus-transfection.com/resources/cell-transfection-database/

Tips to increase cell viability of sensitive cells

- Replace medium 4 h after transfection.
- Check that silencing the target gene does not affect cell viability.

Use appropriate controls

- Positive control: housekeeping gene (GAPDH or HPRT).
- Negative control: mismatch, scramble or non-targeting sequence.
- Be cautious with fluorescently labeled siRNA: 20 to 30 nM are needed to detect a signal, while only 1 nM can be sufficient for efficient silencing using INTERFERin®.

Good siRNA Transfection Practices

- Store appropriately INTERFERin® (5 ± 3°C). Do NOT freeze INTERFERin®.
- Ensure that cells have been passaged more than twice and less than 20 times prior to transfection.
- Discard overconfluent cells.
- Check the half-lives of the protein and mRNA of interest and measure gene silencing accordingly 24 to 96 h after transfection.
- Regularly check for mycoplasma contaminations.
- Serum quality may drastically affect transfection efficiency. When purchasing a new batch of serum or trypsin, check cell viability as well as transfection efficiency.

Note : INTERFERin® is recommended for siRNA transfection. Please refer to the complete protocol available online at: http://www.polyplus-transfection.com/resources/product-literature/ . Please use jetPRIME® for DNA/siRNA cotransfection.

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