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 seeding volume
24-well	15 000 – 35 000	1 ml
6-well / 35 mm	30 000 – 70 000	2 ml
100 mm / flask 75 cm^2	100 000 – 200 000	4 ml

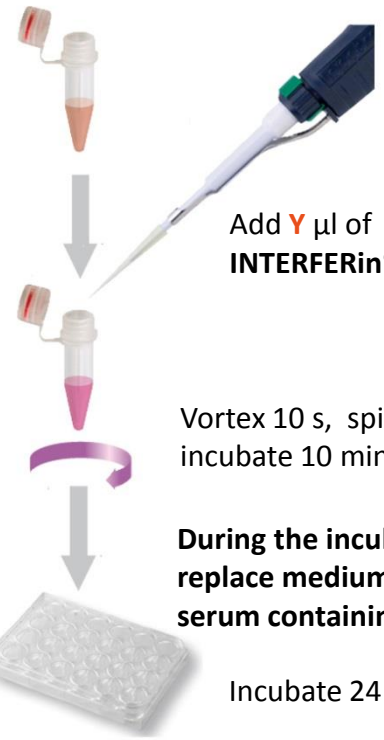
*For suspension cells, please refer to the complete protocol.

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

Add transfection mix
to the cells in serum
containing medium

During the incubation time, replace medium with Z ml 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 (1nM)	Y volume of INTERFERin® reagent	Z volume of serum containing medium
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

DAY 2-3: Measure gene expression

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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Short protocol - Optimization Tips

Use 1 nM siRNA final concentration to reduce off-target effects

Protocol Optimization

- Check our online Cell Transfection Database at:
  http://www.polyplus-transfection.com/resources/cell-transfection-database/
- 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

Tips to increase cell viability of sensitive cells

- 4h after transfection, add medium up to the volume **V** as indicated at **DAY 0**.
- Replace medium after 4 h
- 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 is sufficient for efficient silencing using INTERFERin®

Good siRNA Transfection Practices

- Store appropriately INTERFERin® (4°C) and the siRNA. 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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Contact us:
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