# INTERFERin® transfection reagent

## Short protocol – siRNA transfection

RNA INTERFERENCE

## Day 0: Cell seeding

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

Quantities per well, dish or flask

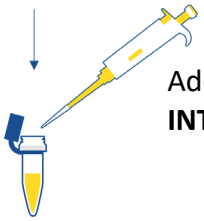
| Culture vessel | Number of cells* | V = volume of medium during transfection |
|---|---|---|
| 96-well | 2 500 – 7 500 | 0.2 mL |
| 24-well | 15 000 – 35 000 | 1 mL |
| 12-well | 30 000 – 70 000 | 2 mL |
| 6-well / 35 mm | 100 000 – 200 000 | 4 mL |
| 100 mm / flask 75 $cm^2$ | 750 000 – 1.25 x $10^6$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

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

## Day 1: Transfection = 1 nM siRNA

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

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

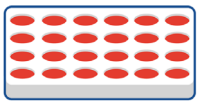


Add **Y** µL of **INTERFERin®** reagent

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

**During the incubation time, replace the cell growth medium with Z mL of fresh medium**

Add transfection mix to the cells



Incubate 24 to 72 h

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 growth 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 |

*in final volume of culture.

## Day 2-3: Analyze gene silencing

See back page for optimization tips

Download complete protocol on https://myaccount.polyplus-transfection.com/

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# INTERFERin® transfection reagent

## Short protocol – Optimization tips

RNA INTERFERENCE

## Protocol Optimization

- The siRNA final concentration may range from 1 to 50 nM depending on the cells and the target gene.
- 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: siRNA against housekeeping genes/fluorescently labelled siRNA.
- 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 when creating your account online at: https://myaccount.polyplus-transfection.com/.
Use jetPRIME® for DNA/siRNA co-transfection.

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