INTERFERin® Transfection Reagent

Short Protocol – siRNA Transfection

Day 0: Cell Seeding

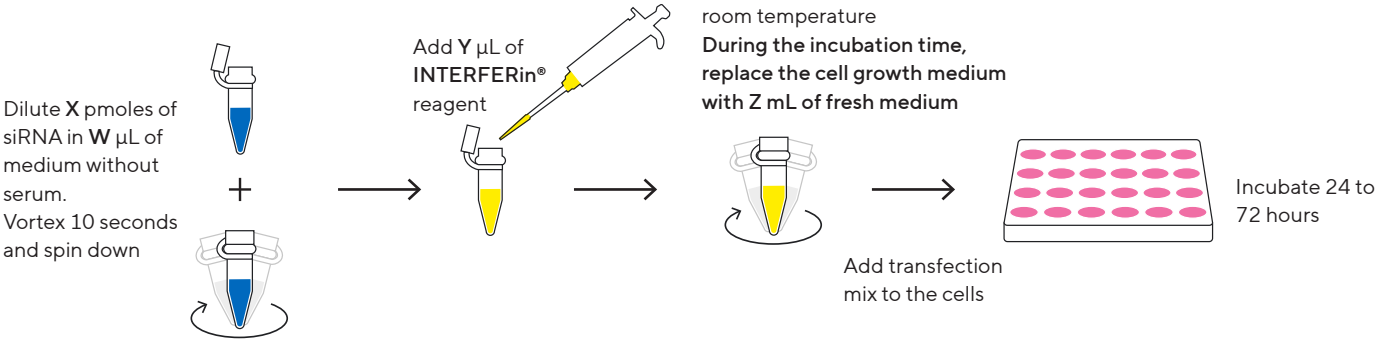
- Seed cells in **V** mL of serum containing medium according to the table below

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

Quantities per well, dish or flask.
*For suspension cells, please refer to the complete protocol.

Day 1: Transfection = 1 nM siRNA

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



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

Quantities per well, dish or flask.
*in final volume of culture.

Day 2 – 3: Analyze Gene Silencing

See back page for optimization tips.

Download complete protocol on sartorius.com

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.

Tips to Increase Cell Viability of Sensitive Cells
- Replace medium 4 hours 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 hours 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.

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