

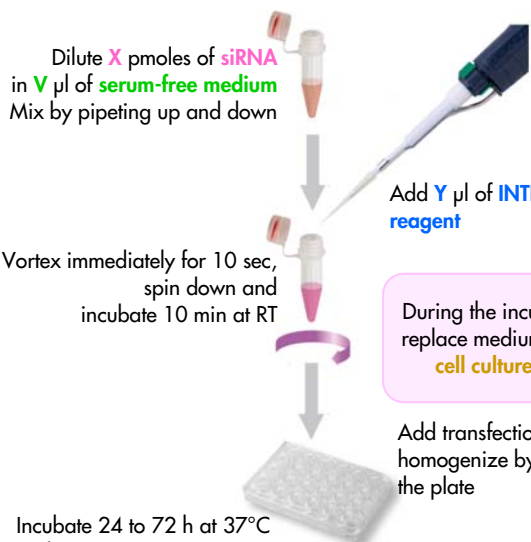

Cell seeding: Cells 30 to 50% confluent at time of transfection

Seed cells in **X** ml of growth medium according to the following table

Culture vessel	Quantities per well	
	Number of cells	X volume of growth medium
24-well	25 000 ± 10 000	1 ml
3.5 cm / 6-well	150 000 ± 50 000	4 ml

On the day of transfection:

Dilute **X** pmoles of **siRNA**
in **V** μ l of **serum-free medium**
Mix by pipeting up and down



Add **Y** μ l of **INTERFERin™ reagent**

During the incubation time,
replace medium with **C** (ml)
cell culture medium

Add transfection mix to the cells,
homogenize by gently swirling
the plate

Incubate 24 to 72 h at 37°C
and measure gene expression

Add per well for 1 nM siRNA

Culture vessel	X amount of siRNA	V volume of serum-free medium	Y volume of INTERFERin™ reagent	C volume of cell culture medium
24-well	0.6 pmoles (8.4 ng)	100 μl	2 μl	0.5 ml
3.5 cm/ 6-well	2.2 pmoles (31 ng)	200 μl	8 μl	2 ml



To ensure optimal use of **INTERFERin™**, we would like to draw your attention to these key points:

➤ **siRNA concentration:**

-1 nM siRNA to reduce off-target effects

The siRNA concentration may need to be adjusted for your cell type. We do not recommend using concentrations above 50 nM and below 0.05 nM.



Cell confluency: between 30 and 50%



Positive Transfection Controls:

- GAPDH siRNA
- Cyclophilin B



Negative Transfection Controls:

- **mismatch siRNA** derived from your targeted sequence
- **non-targeting siRNA** that does not affect any gene in the cell (available from siRNA providers)



Controls to be cautious with:

- **Fluorescent siRNA:** Use 25 nM siRNA
At 1 nM the emitted fluorescence remains below detection level. Please note that this amount does not correlate with the 1 nM siRNA required usually for efficient silencing using **INTERFERin™**.

- siRNA validation using co-transfection:

Use **jetPRIME™** transfection reagent

For personalized support, please contact us at:
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