

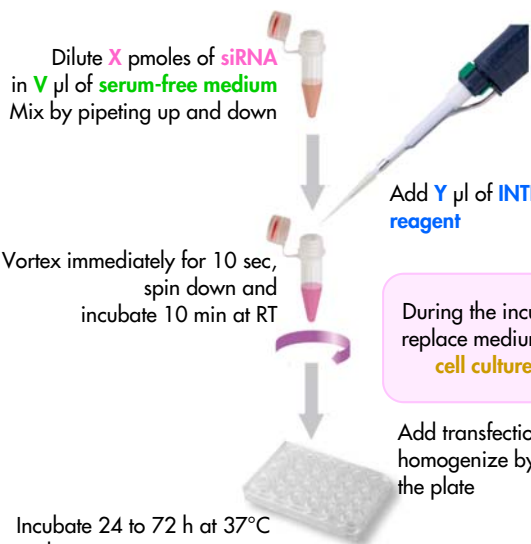

**Cell seeding: Cells 30 to 50% confluent at time of transfection**

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

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

**On the day of transfection:**

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



Add **Y**  $\mu$ 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	<b>X</b> amount of <b>siRNA</b>	<b>V</b> volume of <b>serum-free medium</b>	<b>Y</b> volume of <b>INTERFERin™ reagent</b>	<b>C</b> volume of <b>cell culture medium</b>
24-well	<b>0.6 pmoles</b> (8.4 ng)	<b>100 <math>\mu</math>l</b>	<b>2 <math>\mu</math>l</b>	<b>0.5 ml</b>
3.5 cm/ 6-well	<b>2.2 pmoles</b> (31 ng)	<b>200 <math>\mu</math>l</b>	<b>8 <math>\mu</math>l</b>	<b>2 ml</b>



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:  
[support@polyplus-transfection.com](mailto:support@polyplus-transfection.com)

[www.polyplus-transfection.com](http://www.polyplus-transfection.com)