

Tips for successful *in vitro* RNAi experiments

INTERFERin™ is an easy-to-use reagent that allows efficient delivery of siRNA to cells and robust gene silencing using nanomolar concentrations of siRNA.

The protocol for **INTERFERin™** could hardly be much simpler; it is fast and easy, whether you use a forward or a reverse protocol. In addition, you can keep your cells in serum and antibiotics during transfection.

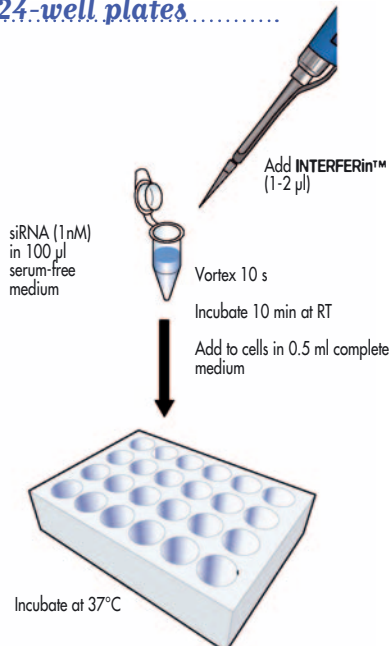
Prepare your cells

- Use healthy cells, preferably at low passage.
- Plate cells at 30 to 50% confluency, one day before transfection.

Prepare the siRNA

- When using low siRNA concentrations, adapt the concentration of the siRNA stock solution accordingly. Prepare a working aliquot allowing you to pipet accurate volumes.

Transfection protocol in 24-well plates



Transfection tips

- Vortex **INTERFERin™** before use. Ensure the reagent was kept refrigerated at 4 °C.
- Do not incubate the siRNA/**INTERFERin™** complexes longer than 30 min.
- For small volumes of **INTERFERin™**, dilute the reagent 1/5 in water and dispense.
- Gently swirl the plate to homogenize after transfection.

Next day

- No need to change medium the day after transfection for standard cell lines. For sensitive cells, replace the medium 4 to 6 hours after transfection or the next morning.

Tips to increase siRNA silencing

- Optimize amount of siRNA used and volume of **INTERFERin™**. For primary cells, use higher concentrations of siRNA (*i.e.* 10, 20 or even 40 nM).
- Use Opti-MEM® to prepare the **INTERFERin™**/siRNA complexes.
- Reduce the volume of medium on the cells by half.
- Centrifuge the plate at 280 g for 5 min and replace medium after 4 hours.

Your gene of interest

- Check the expression profile of your gene.
- Check the turnover of your protein of interest and the half-life of your mRNA (if known) to determine the best time point for analysis.
- Check various time points after transfection namely at 24, 48, 72 and 96 hours after transfection.
- Determine gene silencing efficiency at the RNA level and at the protein level.

Check your siRNA

- Use high quality desalted siRNA.
- Validate your siRNA sequence (for example in a co-transfection assay).
- Use a standard control such as a housekeeping gene or GAPDH siRNA.
- Use a commercially available negative control such as scrambled siRNA.
- Verify siRNA concentration and annealing.
- Ensure the siRNA has been stored properly.

Tips to reduce toxic effects on primary cells

- Replace medium after 4 to 12 hours.
- Reduce the volume of **INTERFERin™**.
- Check that the target gene is not involved in cell viability.

Reagent	Cat. N°	Size	Number of transfections in 24-well plates
INTERFERin™	409-01	0.1 ml	50-100
	409-05	0.5 ml	250-500
	409-10	1 ml	500-1000
	409-50	5 x 1 ml	2500-5000

Bulk quantities are available upon request.

For more information, please contact our technical support:
support@polyplus-transfection.com