Guidelines for successful siRNA transfection using INTERFERin®

Good siRNA transfection practices

- Use a siRNA against a housekeeping gene (GAPDH, cyclophylin B) as a positive control. Use a commercially available negative control (mismatch, non-targeting). Avoid fluorescent siRNA controls when working at low siRNA concentration since high siRNA concentration is required for detection (20-50 nM).

- Use high quality desalted siRNA and verify siRNA concentration and annealing.

- Passage cells at least twice after thawing to allow recovery before transfection, and use cells at low passage number (< 20 passages). Discard cells if they have become overconfluent. Regularly check for contaminants: yeast, bacteria and mycoplasma.

- Check transfection efficiency before purchasing a new batch of serum or trypsin.

- Store appropriately INTERFERin® (4°C, do not freeze) and the siRNA.

Know the target gene

- Design the siRNA sequence as efficiently as possible by comparing several algorithms. The better the siRNA, the lower the concentration needed for high silencing.

- Check the half-lives of the protein and of the mRNA of interest to determine the best time point of analysis, or analyze at 24, 48, 72 and 96 hours after transfection.

- Analyze gene silencing at both the mRNA and the protein level.

Transfection tips

- The day before transfection, seed the cells to obtain 30-50% confluency at the time of transfection. Perform transfection in regular growth medium (INTERFERin® is compatible with serum and antibiotics).

- Prior to transfection, dilute the siRNA in medium without serum (ex.Opti-MEM™) first, and then add the INTERFERin® reagent.

- When using low siRNA concentrations, adapt the concentration of the siRNA stock solution allowing you to pipet accurate volumes. Proceed the same way for small volumes of INTERFERin®: dilute 1 to 5 in sterile water.

- Do not incubate the siRNA with INTERFERin® for more than 30 minutes.

Tips to increase siRNA silencing

- Use higher siRNA concentration (10, 20 or even 50 nM) and higher volume of INTERFERin®.

- Perform transfection in half as much growth medium.

- Centrifuge the plate at 180 g for 5 min and replace medium after 4 hours.

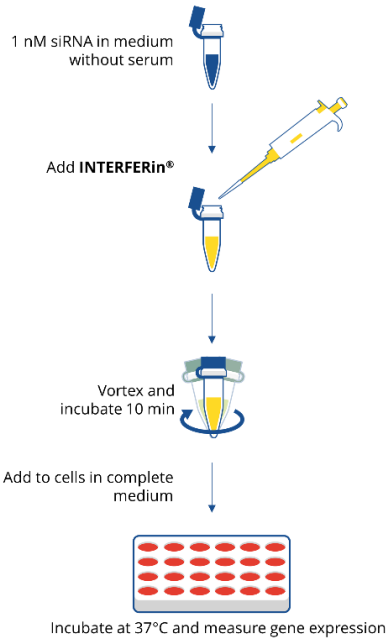
Tips to increase cell viability

- Replace medium after 4 to 6 h.

- Reduce the volume of INTERFERin®.

- Decrease siRNA concentration.

Transfection protocol



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