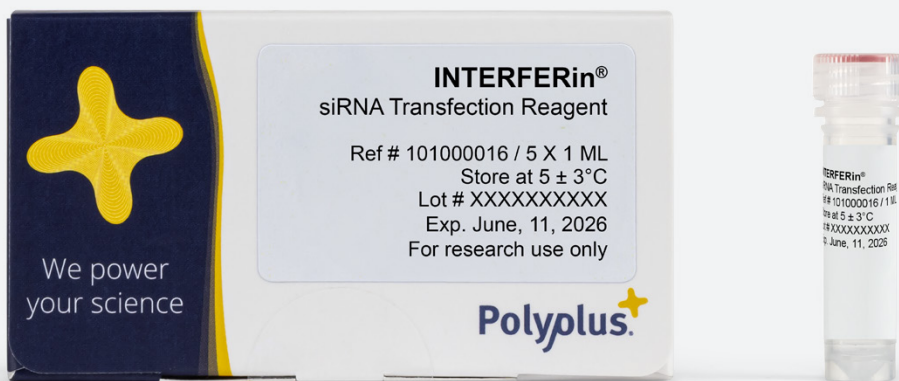


INTERFERin®



Technical Note

Scope

- Provides over 90% gene silencing
- Can be used in a wide variety of adherent and suspension cells
- Suitable for transfection miRNA and other oligonucleotides
- Gentle mode of action for more robust data and excellent cell viability

Good siRNA Transfection Practices

- Use a siRNA against a housekeeping gene (GAPDH, cyclophilin 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.

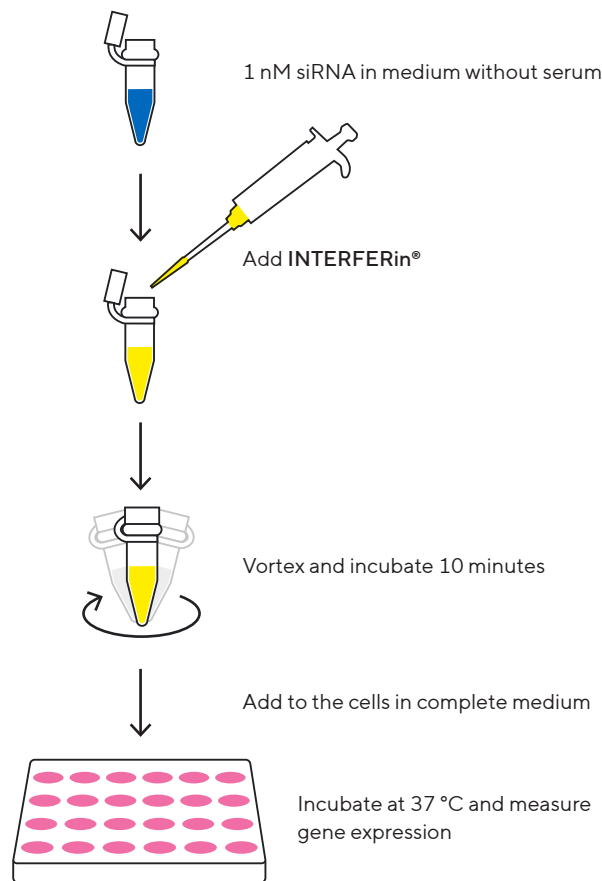
Tips to Increase siRNA Silencing

- 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 minutes and replace medium after 4 hours.

Tips to Increase Cell Viability

- Replace medium after 4 to 6 hours.
- Reduce the volume of INTERFERin®.
- Decrease siRNA concentration.

Transfection Protocol



Opti-MEM is a trademark of Invitrogen Corporation.

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