



HTS applications

INTERFERin™

siRNA transfection reagent

- > **Protocols adapted for HTS**
- > **Great silencing at 1 nM siRNA** for better results and cost savings
- > **90% gene silencing** in a variety of cells
- > **Gentle** mode of action for more robust data and excellent cell viability
- > **Compatible** with serum and antibiotics

Highly efficient knockdown in 96- and 384-well plates with 1 nM siRNA

INTERFERin™ transfection reagent enables highly efficient siRNA transfection for high-throughput applications with either standard, reverse or batch protocols (Fig. 1). Using low nanomolar siRNA concentrations avoids unwanted toxic and off-target effects associated with reagents requiring higher siRNA concentrations^{1,2}, resulting in more robust data. For automated approaches INTERFERin™ may be diluted for convenience. INTERFERin™ is compatible with serum and antibiotics, thereby eliminating medium changes.

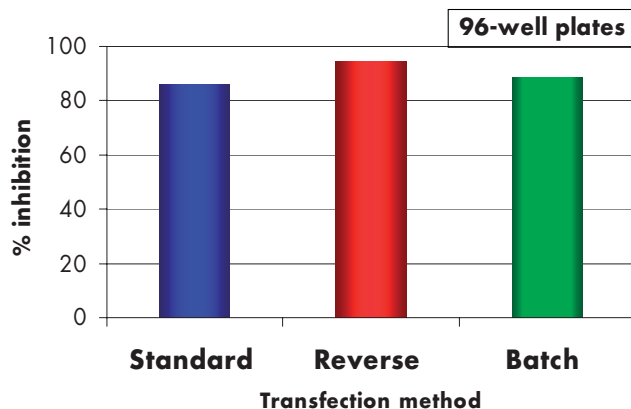


Figure 1. Inhibition of luciferase expression 48 h after transfection in A549 cells stably expressing the luciferase gene with INTERFERin™ using indicated transfection protocols. Cells were transfected in the presence of serum with 1 nM Luciferase siRNA (GL3Luc).

Reverse transfection protocol

Highly efficient gene silencing results are obtained with the reverse transfection protocol (Fig. 1). With the reverse transfection method, the siRNA/ INTERFERin™ complexes are prepared directly in the wells and the cells are added subsequently (Fig. 2). One nanomolar siRNA is recommended as a starting concentration for silencing experiments.

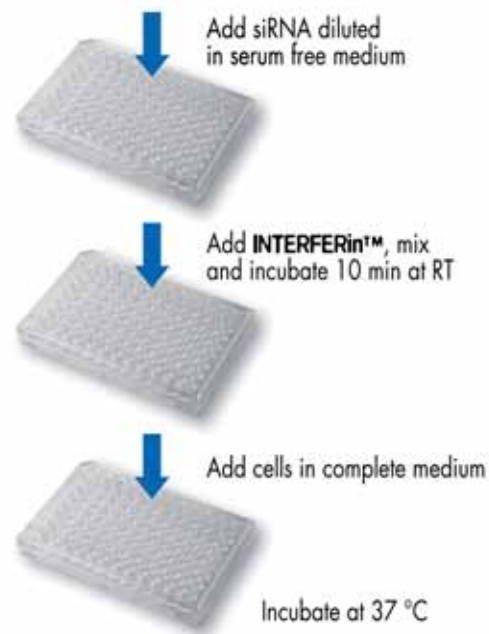


Figure 2. INTERFERin™ reverse transfection protocol for 96-well plates at 1 nM final siRNA concentration.

The reverse transfection protocol does not affect cell viability; cells look healthy 48 h after transfection (Fig. 3).



Figure 3. Cell morphology of A549-GL3Luc 48 h after reverse transfection in 96-well plates. Cells were transfected with 1 nM luciferase siRNA (GL3Luc) complexed with 5 µl of diluted INTERFERin™.

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Efficient endogenous gene silencing

Effective knockdown is observed in a variety of cell lines and primary cells when silencing endogenous genes. For example, 85 to 95% silencing of GAPDH gene expression is observed in selected carcinoma cell lines (Fig. 4).

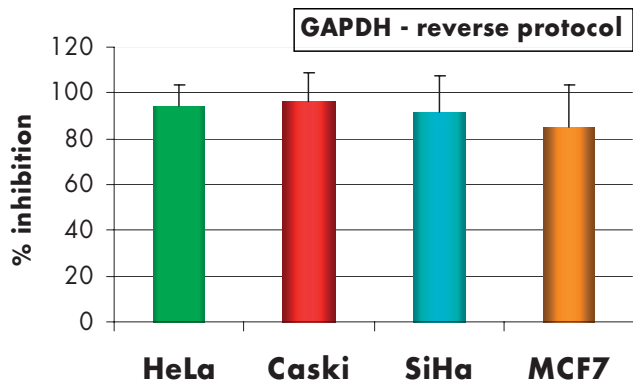


Figure 4. Endogenous GAPDH gene silencing with 1 nM GAPDH siRNA using the reverse transfection protocol with INTERFERin™ in 96-well plates. GAPDH gene silencing efficiency was determined after 48 h by branched DNA mRNA quantification.

| Product | Cat N° | Reagent |
|-------------|----------|----------|
| INTERFERin™ | 409-01 | 0.1 ml |
| | 409-05 | 0.5 ml |
| | 409-10 | 1 ml |
| | 409-50 | 5 x 1 ml |
| | 409B-010 | 10 ml |

1 ml of INTERFERin™ is sufficient to perform 1000-1200 transfections in 96-well plates or 2000 transfections in 384-well plates.

Larger volumes available upon request.

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

Batch transfection

INTERFERin™ is also efficient in batch transfection, for homogenous screening of a specific target. This method is particularly well-suited for drug screening. With this very simple protocol, the siRNA, INTERFERin™ and the cells are mixed sequentially and distributed to the wells on the same day (Fig. 5). Approximately, 90% silencing efficiency is reached with the batch transfection protocol at 1 nM siRNA (Fig. 1).

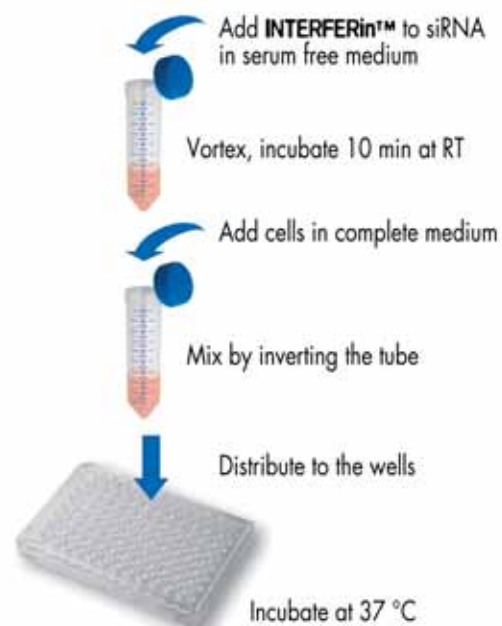


Figure 5. INTERFERin™ batch transfection protocol for 96-well plates at 1 nM final siRNA concentration.

- Semizarov D. *et al.* (2003) Specificity of short interfering RNA determined through gene expression signatures. *Proc. Natl Acad. Sci. USA* **100**: 6347.
- Persengiev S.P. *et al.* (2004) Nonspecific, concentration-dependent stimulation and repression of mammalian gene expression by small interfering RNAs. *RNA* **10**:12.