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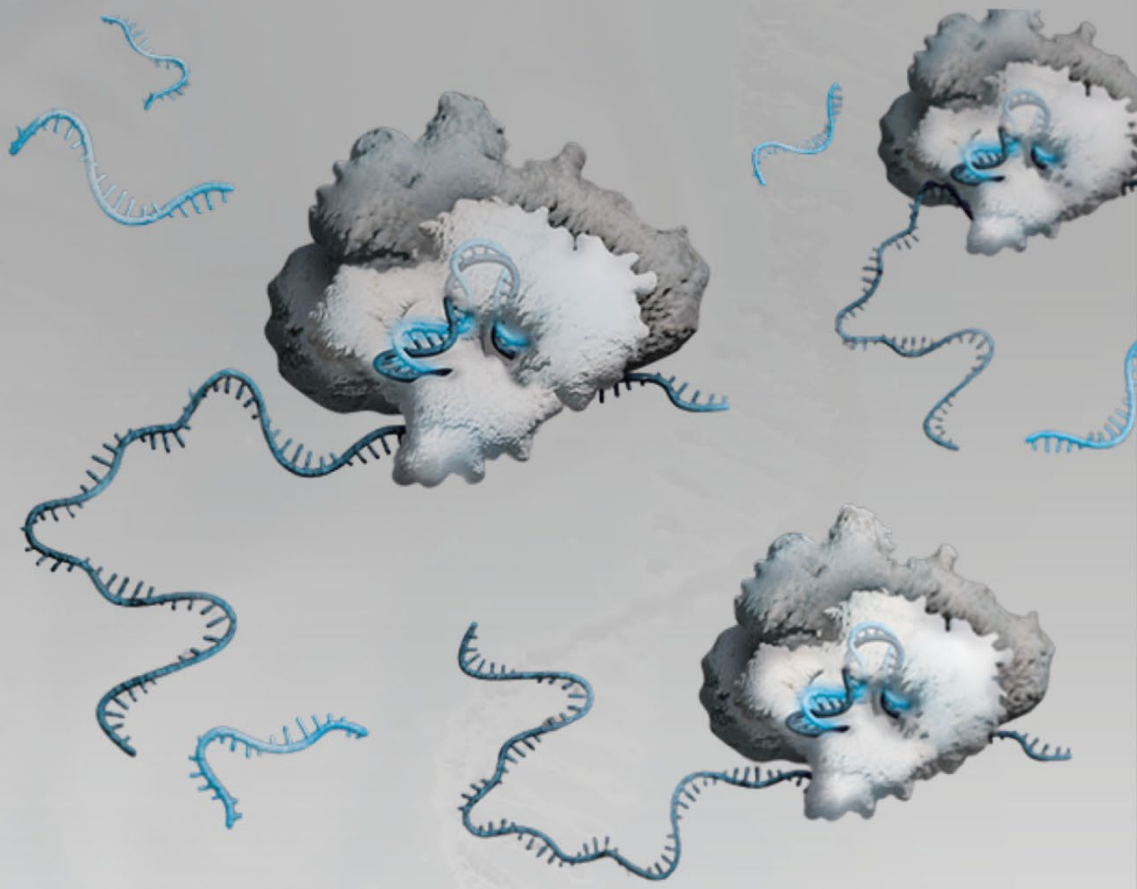


siPOOL™ Transfection Protocol

Concentration, efficacy and specificity

Efficacy and specificity of siPOOL-based knock-down is dependent on the siRNA concentration in the cells. Optimal transfection conditions will deliver an amount of siRNAs into the cytoplasm sufficient for a saturating on-target knock-down, but avoiding an overload that may lead to unwanted off-target effects. The efficacy of siRNA delivery is largely dependent on the cell type and transfection reagent used.

Cells

This protocol is optimized for the transfection of adherent mammalian cell lines (A549, MCF7, HeLa or HEK293). Many types of primary cells, embryonic stem cells and certain cell lines (for e.g. MDCK, BJ or GH3) are significantly harder to transfect. Optimal transfection conditions for these cells may require testing of different transfection reagents and frequently higher siPOOL concentrations. (For advice and help on setting up a transfection experiment please contact info@sitools.de).

Transfection reagents

There are many commercially available transfection reagents which vary in their efficacy and toxicity in different cell types and cell lines. For many adherent cell lines, Lipofectamine® RNAiMAX (Thermo Fisher) gives close to optimal results and is, therefore, recommended for starting transfection optimization of siPOOLs.

Guidelines and parameters

The 3 most important parameters defining transfection efficacy, toxicity and assay compatibility are a) siRNA concentration, b) transfection reagent concentration, and c) cell seeding density. For adaption of the basic protocol below, follow these general guidelines:

a) siRNA concentration: keep the siPOOL concentration as low as possible. For optimal results, quantify silencing efficacy for a test gene by qPCR (e.g. GAPDH) or phenotypic readout (e.g. PLK1) to establish the lowest siPOOL concentration required for maximum gene silencing. For most cells, this concentration is between 1 nM and 3nM.

b) Transfection reagent concentration must be adapted to cell seeding density. High reagent concentration and low cell seeding density will lead to target gene-independent toxicity that may mask target gene-specific phenotypes.

c) Adapt your cell seeding density to your assay protocol. The protocol below is intended for analysis 24 hours post transfection as recommended by qPCR analysis of target gene expression.

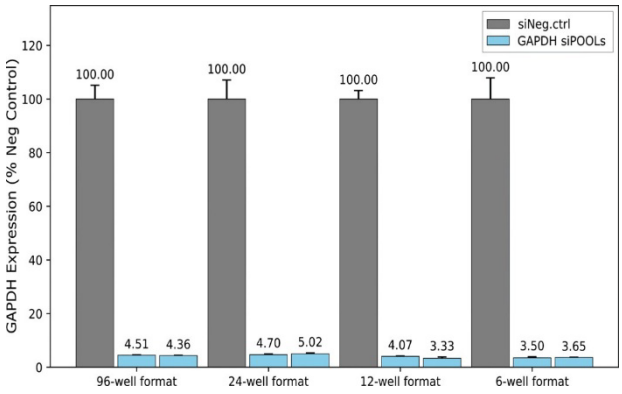


Figure 1: siPOOL-based knock-down efficacy in different plate formats. A549 cells were transfected in duplicates with the GAPDH siPOOL according to our recommended reverse transfection protocol (see Snapshot protocol below) in 96-, 24-, 12- and 6-well formats. GAPDH knock-down was assessed 24 hours after transfection by qPCR (average ± SD).

Results show successful and consistent knock-down of GAPDH (>94%) in all plate formats.

Forward and reverse transfection:

The complex of siPOOL and transfection reagent may be added onto adherent cells (forward transfection) or can be pre-mixed with cells prior during the seeding process (reverse transfection).

During **forward transfection**, cells are classically seeded 24 hours prior to transfection. This approach may be better suited for certain screening procedures and may have lower toxicity in some cell lines. Cells will have to be seeded at lower density to reach the same confluency at time of analysis.

Reverse transfection is the protocol of choice for most applications as it frequently yields better transfection efficacy, is more time efficient, and allows for longer assay duration. The snapshot protocol below describes the transfection of siPOOLs using reverse transfection with the Lipofectamine® RNAiMAX reagent.

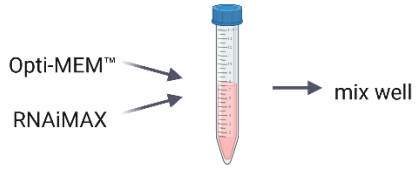
Snapshot protocol: siPOOL reverse transfection

Additional reagents required:
Transfection reagent: Lipofectamine® RNAiMAX
Serum-free medium: Opti-MEM™
Cell culture medium (containing serum)

1. Prepare the RNAiMAX solution [A) B)]

Plate format	Opti-MEM™	Lipofectamine® RNAiMAX	Total volume
96-well	12.25 µL	0.25 µL	12.5 µL
24-well	49 µL	1 µL	50 µL
12-well	98 µL	2 µL	100 µL
6-well	196 µL	4 µL	200 µL

The volumes can be scaled up and down according to your transfection format.

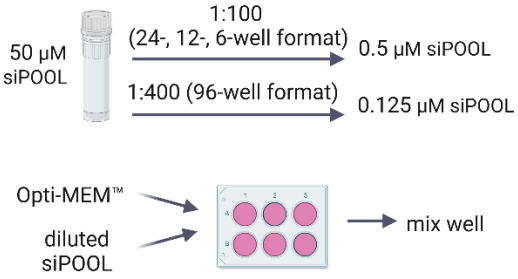


2. Prepare siPOOL working solution [A) B)]

Dilute the siPOOL in H_2O to a working conc. of 0.5 uM (24-, 12-, 6-well format) or 0.125 uM (96-well format).

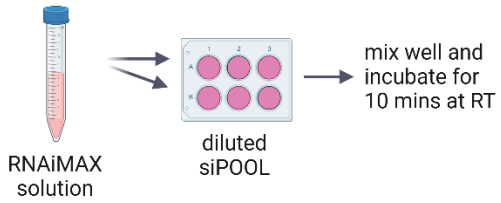
Pipette the following into each transfection well.

Plate format	Opti-MEM™	diluted siPOOL	Total volume
96-well	9.5 µL	3 µL	12.5 µL
24-well	47 µL	3 µL	50 µL
12-well	94 µL	6 µL	100 µL
6-well	192 µL	8 µL	200 µL



3. Combine RNAiMAX solution and diluted siPOOL

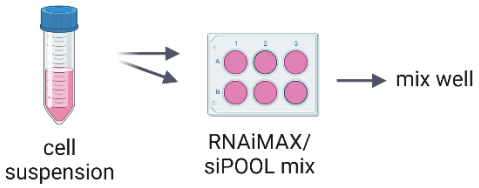
Transfer the appropriate amount of the previously prepared RNAiMAX solution (see Step 1) to each well with diluted siPOOL.



4. Seed cells [B)]

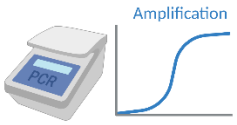
Seed the indicated number of cells/well in normal cell growth medium.

Plate format	Cells/well ($x10^5$)	Volume (µL)
96-well	0.125	100
24-well	0.5	400
12-well	1	800
6-well	2	1600



5. Validate knock-down

Gene-specific knock-down can be validated by qPCR analysis 24 hours later.



General considerations:
A) Depending on the type of experiment, choose the appropriate number of biological replicates. The RNAiMAX solution (1) and the siPOOL working solution (2) can be prepared in master mixes by multiplying the specified volumes by the number of replicates (+ excess).
B) Consider including a negative control (non-targeting siPOOL) to ensure on-target specificity of knock-down phenotype.
C) Transfection in cell culture dishes: reactions can be scaled up for transfection optimization in dish format (consider manufacturer's recommendations for transfection reagents).

For technical inquiries and further information please contact us: **info@sitools.de**

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