

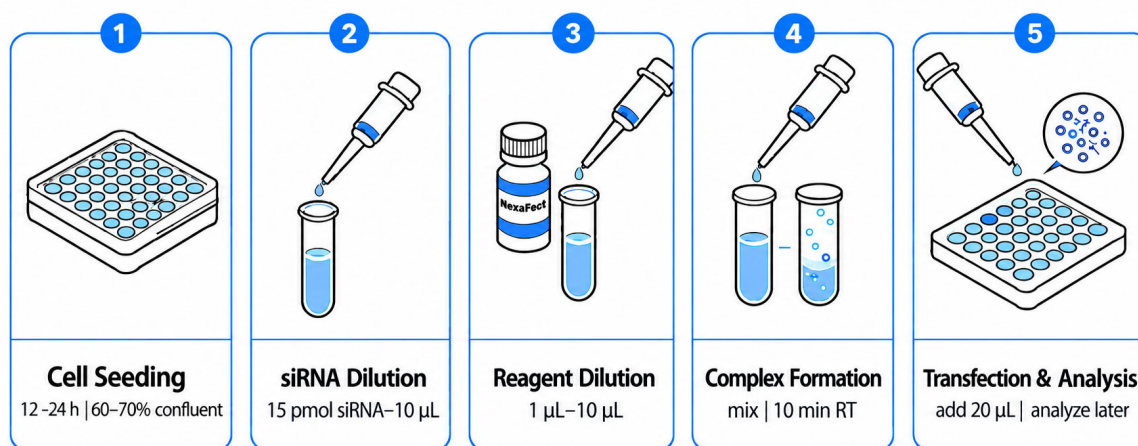
NexaFect™ siRNA Transfection Reagent Protocol

NexaFect™ siRNA Transfection Reagent is a high-performance transfection reagent specifically designed for efficient siRNA delivery. It effectively complexes and protects siRNA from nuclease degradation, enabling rapid and efficient delivery of siRNA into eukaryotic cells.

NexaFect™ siRNA Transfection Reagent is suitable for siRNA transfection in a broad range of primary cells and established cell lines. It provides high gene-silencing efficiency with consistent performance and can achieve effective gene knockdown in cell culture media both in the presence and absence of serum.

siRNA Transfection Procedure

The following protocol is optimized for transfection of one well of a 24-well plate. Transfection parameters for various culture vessel formats are detailed in Table 1.



1. Cell Seeding

Seed 50,000 cells per well and culture for 12–24 hours. At the time of transfection, cells should be approximately 60–70% confluent.

2. Dilution of siRNA

Dilute 15 pmol siRNA with serum-free medium (e.g., Opti-MEM™ I Reduced Serum Medium) to a final volume of 10 µL.

3. Dilution of NexaFect™ siRNA Transfection Reagent

Dilute 1 µL of NexaFect™ siRNA Transfection Reagent with 9 µL of serum-free medium to a final volume of 10 µL.

4. Preparation of siRNA–Reagent Complexes

Combine the diluted siRNA with the diluted NexaFect™ siRNA Transfection Reagent. Mix gently by pipetting and incubate at room temperature for 10 minutes to allow siRNA–reagent complexes to form.

5. Transfection and Analysis

Add the 20 µL siRNA–reagent complex to one well of cells and mix gently. Continue incubation for 18–48 hours, then evaluate knockdown efficiency or target gene expression.

No medium change is required during the transfection procedure.

Optimization of siRNA Transfection

1. Cell Density

For optimal transfection, cells should be healthy, actively growing, and in the logarithmic growth phase. Cell confluency should be $\geq 60\%$ at the time of transfection.

2. Recommended Reagent-to-siRNA Ratio

NexaFect™ siRNA Transfection Reagent (μL) : siRNA (pmol) = 0.02:1 to 0.15:1

For initial experiments, a ratio of 0.067:1 is recommended.

3. If Knockdown Efficiency Is Low

If the cells remain healthy but gene knockdown efficiency is low, gradually increase the amount of NexaFect™ siRNA Transfection Reagent. Do not exceed a reagent-to-siRNA ratio of 0.15:1.

4. If Cytotoxicity Is Observed

If significant cell shrinkage, detachment, floating cells, or cell death is observed after transfection, reduce the amount of NexaFect™ siRNA Transfection Reagent. A reagent-to-siRNA ratio near 0.02:1 may be used to reduce cytotoxicity.

5. Experimental Controls

When optimizing the reagent-to-siRNA ratio, keep the complex formation conditions and incubation time constant to minimize the influence of other variables. For suspension cells, ensure that the cells are well dispersed and avoid cell clumping, as aggregation may reduce transfection efficiency. It is recommended to include a negative-control siRNA in the experiment.

Table 1. Transfection Parameters Optimized for Various Culture Vessel Formats

Culture Vessel	Surface Area per Well	Culture Medium Volume per Well	Number of Cells Seeded per Well	Volume of siRNA-Reagent Complex per Well	NexaFect™ siRNA Transfection Reagent Amount per Well	siRNA Amount per Well
96-Well Plate	0.3 cm ²	200 μL	10,000	10 μL	0.5 μL	7.5 pmol
24-Well Plate	2.0 cm ²	500 μL	50,000	20 μL	1.0 μL	15 pmol
12-Well Plate	4.0 cm ²	1 mL	100,000	40 μL	2.0 μL	30 pmol
6-Well Plate	10.0 cm ²	2 mL	200,000	100 μL	4.0 μL	60 pmol

Store at 2–8°C. DO NOT FREEZE.

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