

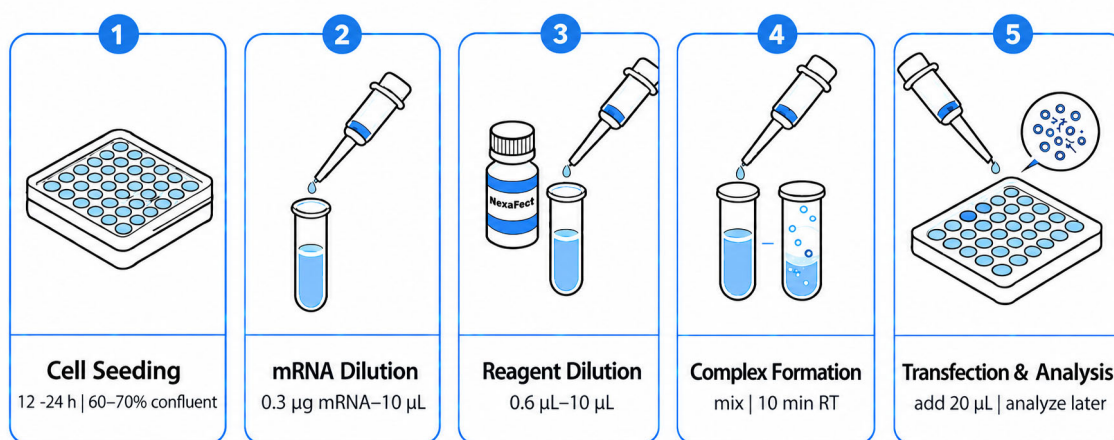
NexaFect™ mRNA Transfection Reagent Protocol

NexaFect™ mRNA Transfection Reagent is a high-performance transfection reagent specifically designed for efficient mRNA delivery into a broad range of adherent and suspension cell types. It delivers mRNA directly into the cytoplasm for protein expression, bypassing transcriptional regulation and the requirement for nuclear entry.

NexaFect™ mRNA Transfection Reagent is suitable for applications requiring short-term protein expression and provides high transfection efficiency with consistent performance across a wide variety of commonly used and difficult-to-transfect cell lines, as well as primary cells and stem cells.

mRNA 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 mRNA

Dilute 0.3 µg of mRNA with serum-free medium (e.g., Opti-MEM™ I Reduced Serum Medium) to a final volume of 10 µL.

3. Dilution of NexaFect™ mRNA Transfection Reagent

Dilute 0.6 µL of NexaFect™ mRNA Transfection Reagent with 9.4 µL of serum-free medium to a final volume of 10 µL.

4. Preparation of mRNA–Reagent Complexes

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

5. Transfection and Analysis

Add the 20 µL mRNA–reagent complex to one well of cells and mix gently. Continue incubation for 12–48 hours, then evaluate transfection efficiency or target gene expression.

No medium change is required during the transfection procedure.

Optimization of mRNA 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-mRNA Ratio

NexaFect™ mRNA Transfection Reagent (μL) : mRNA (μg) = 1:1 to 5:1

For initial experiments, ratios of 2:1 or 3:1 are recommended.

3. If Transfection Efficiency Is Low

If the cells remain healthy but fluorescence intensity or target gene expression is low, gradually increase the amount of NexaFect™ mRNA Transfection Reagent. Do not exceed a reagent-to-mRNA ratio of 5:1.

4. If Cytotoxicity Is Observed

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

5. Experimental Controls

When optimizing the reagent-to-mRNA ratio, keep the complex formation conditions and incubation time constant to minimize interference from other variables. Ratios outside the recommended range may result in reduced transfection efficiency or increased cytotoxicity. For suspension cells, ensure that cells are well dispersed and avoid cell clumping, as aggregation may reduce transfection efficiency.

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 mRNA–Reagent Complex per Well	NexaFect™ mRNA Transfection Reagent Amount per Well	mRNA Amount per Well
96-Well Plate	0.3 cm ²	200 μL	10,000	10 μL	0.2 μL	0.1 μg
24-Well Plate	2.0 cm ²	500 μL	50,000	20 μL	0.6 μL	0.3 μg
12-Well Plate	4.0 cm ²	1 mL	100,000	40 μL	1.2 μL	0.6 μg
6-Well Plate	10.0 cm ²	2 mL	200,000	100 μL	2.4 μL	1.2 μg
60-mm Dish	20.0 cm ²	5 mL	400,000	200 μL	4.8 μL	2.4 μg
100-mm Dish	60.0 cm ²	15 mL	800,000	600 μL	14.4 μL	7.2 μg

Store at 2–8°C. DO NOT FREEZE.

For Research Use Only. Not intended for therapeutic or diagnostic use in humans or animals.