

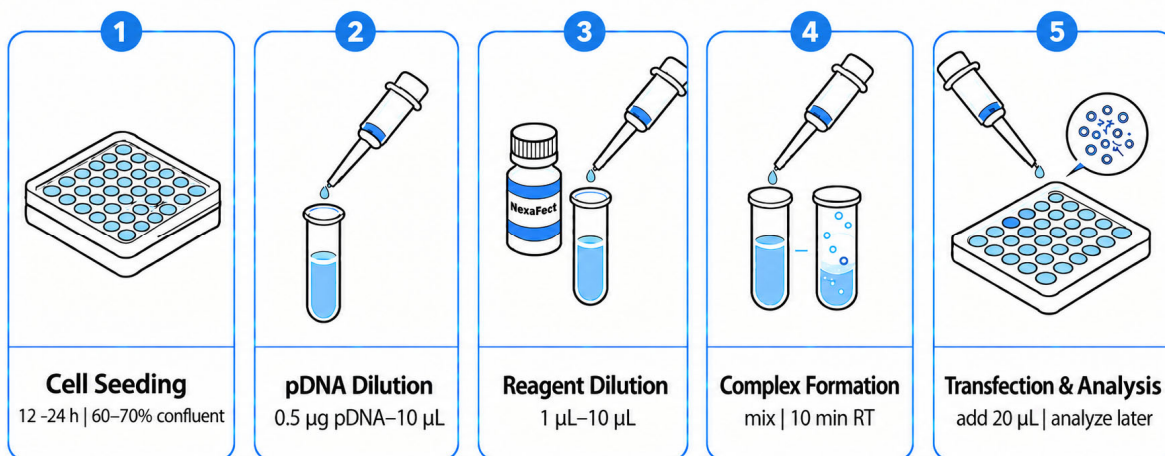
NexaFect™ DNA Transfection Reagent Protocol

NexaFect™ DNA Transfection Reagent is a high-performance transfection reagent designed for efficient intracellular delivery of plasmid DNA (pDNA) and subsequent gene expression.

NexaFect™ DNA Transfection Reagent can be used for plasmid DNA transfection, transient transfection, and generation of stable cell lines. It is suitable for a broad range of adherent cell lines and provides high transfection efficiency with excellent reproducibility in commonly used cell lines, including HeLa, 293T, CHO, Vero E6, LX-2 and MDA-MB-231 cells.

Plasmid DNA 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 Plasmid DNA

Dilute 0.5 µg of plasmid DNA with serum-free medium (e.g., Opti-MEM™ I Reduced Serum Medium) to a final volume of 10 µL.

3. Dilution of NexaFect™ DNA Transfection Reagent

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

4. Preparation of pDNA–Reagent Complexes

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

5. Transfection and Analysis

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

No medium change is required during the transfection procedure.

Optimization of Plasmid DNA 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-pDNA Ratio

NexaFect™ DNA Transfection Reagent (μL) : plasmid DNA (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™ DNA Transfection Reagent. Do not exceed a reagent-to-pDNA 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™ DNA Transfection Reagent. A reagent-to-pDNA ratio near 1:1 may be used to reduce cytotoxicity.

5. Experimental Controls

When optimizing the reagent-to-pDNA ratio, keep the complex formation conditions and incubation time constant to minimize the influence of other variables. Ratios outside the recommended range may result in reduced transfection efficiency or increased cytotoxicity.

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 pDNA–Reagent Complex per Well	NexaFect™ DNA Transfection Reagent Amount per Well	pDNA Amount per Well
96-Well Plate	0.3 cm ²	200 μL	10,000	10 μL	0.4 μL	0.2 μg
24-Well Plate	2.0 cm ²	500 μL	50,000	20 μL	1.0 μL	0.5 μg
12-Well Plate	4.0 cm ²	1 mL	100,000	40 μL	2.0 μL	1.0 μg
6-Well Plate	10.0 cm ²	2 mL	200,000	100 μL	4.0 μL	2.0 μg
60-mm Dish	20.0 cm ²	5 mL	400,000	200 μL	8.0 μL	4.0 μg
100-mm Dish	60.0 cm ²	15 mL	800,000	600 μL	24.0 μL	12.0 μg

Store at 2–8°C. DO NOT FREEZE.

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