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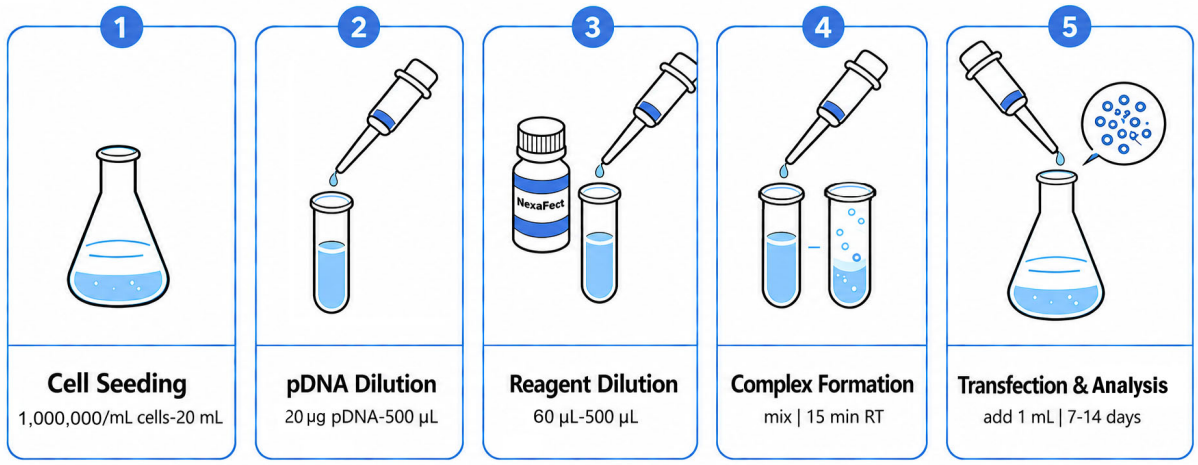
# NexaFect™ LPEI 25K Transfection Reagent (Solution) Protocol

NexaFect™ LPEI 25K Transfection Reagent is a linear polyethylenimine (LPEI) derivative optimized for efficient plasmid DNA delivery and large-scale transient transfection in suspension HEK293 and CHO cells. By combining high transfection efficiency with low cytotoxicity and consistent lot-to-lot performance, it delivers a reliable, cost-effective solution for scalable protein production.

## Plasmid DNA Transfection Procedure

The following protocol is optimized for a 125 mL shake flask. Transfection parameters for other culture vessel sizes are provided in Table 1.




### 1. Cell Seeding

Seed cells at a density of 1,000,000 cells/mL in 20 mL of culture medium. Culture at 37°C and 120 rpm in an atmosphere of 5% $CO_2$.

### 2. Dilution of Plasmid DNA

Dilute 20 µg of plasmid DNA in serum-free medium (e.g., Opti-MEM™ I Reduced Serum Medium) to a final volume of 500 µL.

### 3. Dilution of NexaFect™ LPEI 25K Transfection Reagent

Dilute 60 µL of NexaFect™ LPEI 25K Transfection Reagent in serum-free medium to a final volume of 500 µL.

### 4. Preparation of pDNA–Reagent Complexes

Combine the diluted plasmid DNA and diluted transfection reagent. Mix gently by pipetting and incubate at room temperature for 15 minutes.

### 5. Transfection and Analysis

Add 1.0 mL of the transfection complexes to the cell culture flask. Continue shaking incubation for 7–14 days and assess transfection efficiency.

## Optimization of Plasmid DNA Transfection

Transient transfection conditions can be optimized by adjusting three core variables: cell seeding density, plasmid DNA amount, and transfection reagent amount.

### 1. Plasmid DNA

Always use high-quality, endotoxin-free plasmid DNA.

### 2. Cell Seeding Density

A seeding density of 1,000,000 viable cells per 1 mL of culture medium is recommended and may be fine-tuned according to the growth characteristics of the cell line and the scale of the bioreactor.

### 3. Recommended Reagent-to-pDNA Ratio

NexaFect™ LPEI 25K Transfection Reagent (µL) : pDNA (µg) = 0.5:1 to 4:1.

### 4. If Transfection Efficiency Is Low

If the transfection positivity rate or target protein expression level is low, the transfection reagent ratio may be increased appropriately, up to a maximum of 4:1.

### 5. If Cytotoxicity Is High

If significant cytotoxicity is observed and the viable cell rate decreases substantially, the transfection reagent ratio should be reduced, down to a minimum of 0.5:1.

When the transfection culture period exceeds 7 days, a fed-batch feeding strategy may be used to add an appropriate amount of feed medium, based on cell viability and nutrient consumption status, in order to maintain cell viability, extend the protein expression period, and increase overall product yield.

**Table 1. Transfection Parameters Optimized for Different Culture Vessel Sizes**

| Culture Flask | Cell Density | Culture Medium Volume | Volume of pDNA–Reagent Complex | NexaFect™ LPEI 25K Transfection Reagent Amount | pDNA Amount |
|---|---|---|---|---|---|
| **125 mL** | 1,000,000/mL | 20 mL | 1.0 mL | 60 µL | 20 µg |
| **250 mL** | 1,000,000/mL | 50 mL | 2.5 mL | 150 µL | 50 µg |
| **500 mL** | 1,000,000/mL | 100 mL | 5.0 mL | 300 µL | 100 µg |
| **1.0 L** | 1,000,000/mL | 250 mL | 10.0 mL | 750 µL | 250 µg |

**Store at 2–8°C. DO NOT FREEZE.**

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

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