Frequently Asked Questions (FAQs) and Troubleshooting Guide

1. Low Transfection Efficiency

1.1 Optimize Transfection Parameters
Optimize transfection parameters for each cell type. Extended incubation time: Adjust the incubation time of the transfection complex with cells. The maximum incubation time is 2 hours for cell lines, and 30 minutes for primary cells. Increase ProteanFect transfection complex: Consider increasing the amount of transfection complex to improve transfection efficiency.

1.2 Severe Cytotoxicity Caused by Plasmid DNA
The transfection of pDNA into primary cells, such as primary T cells, can induce cytotoxicity and inflammatory responses. Due to the risk of significant toxicity, pDNA transfection is generally not recommended for primary T cells.

1.3 Use Positive Control
We recommend using a 96-well plate format to optimize transfection conditions for a specific cell type, with EGFP mRNA as the positive control.

1.4 Why endotoxin-free matters for effective pDNA delivery?
Endotoxin (LPS) triggers innate immune pathways and is cytotoxic in immune cell lines (such as Jurkat) and many primary immunocytes. To ensure optimal cell health and transfection efficiency, always use endotoxin-free plasmid DNA for delivery experiments.

1.5 Can I use plasmid DNA without specified endotoxin levels?
We do not recommend using plasmid DNA of unknown endotoxin content. If you are using in-house prepared plasmid DNA, use an endotoxin-free purification kit to achieve ≤ 0.1 EU/μg. For plasmid DNA obtained from third parties, treat the sample with an endotoxin removal column before forming the transfection complex to ensure compatibility with sensitive cell types.

2. Low Cell Viability
Transfected cells may exhibit transient changes in behavior, but typically, viability will be restored by the second day post-transfection.

3. Should I do a suspension transfection or adherent transfection?
Suspension transfection may yield higher efficiency in certain adherent cell lines. We recommend testing both methods to determine the optimal condition.

4. When should I use Reagent C?
Reagent C is not required for most cell lines but may boost efficiency in hard-to-transfect ones. We suggest testing its impact in untested cell lines, both with and without Reagent C.