Lipo3K Transfection Reagent

Introduction

Lipo3K Transfection Reagent is a cationic lipid transfection reagent widely used in cell transfection, which can transfect DNA or RNA (such as siRNA/mRNA) into various types of cells, including adherent cells and suspension cells and even some difficult-to-transfect cells. The transfection reagent is used in the same way as the commonly used Lipofectamine 3000 Reagent, and the transfection efficiency of this transfection reagent has been tested to be comparable to the Lipofectamine® 3000 Reagent.

Compared with Lipo2K, another commonly used lipid transfection reagent, this transfection reagent has higher transfection efficiency and lower cytotoxicity. Compared to Lipo2K, this transfection reagent has a 2-10 fold increase in transfection efficiency, so it is ideal for difficult-to-transfect cells. At the same time, because of the lower cytotoxicity, this reagent does not need to be changed medium after transfection, and the cells can be directly collected for detection 24-48 h after transfection, which is simpler and more convenient. Moreover, a transfection enhancement reagent (Lipo3K-A Reagent) is provided in this kit, which can help plasmid DNA enter the nucleus and greatly increase transfection efficiency. The transfection enhancement reagent is not required for siRNA transfection. In addition, this transfection reagent is not only suitable for transfection of a single plasmid or siRNA, but also for transfection of multiple plasmids or co-transfection of plasmids and siRNAs. Plasmid expression can be detectable 24-48 h after transfection, and siRNA silencing should be achieved 3-5 days after transfection.

When transfecting cells with this reagent, the presence of serum or antibiotics does not affect the transfection efficiency. However, for optimal transfection, it is recommended to use a serum-containing medium without antibiotics for transfection.

Components and Storage

Components	K2705-0.75 mL	K2705-1.5 mL
Lipo3K-A Reagent	0.75 mL	1.5 mL
Lipo3K-B Reagent	0.75 mL	1.5 mL
Store the kit at 4°C, stable for 1 year. Do not freeze.		

Protocol

Use the following procedure to transfect in a 24-well plate. For other plates, the dosage of each

component for transfection is referred to in Table 1.

1. **Cell culture：**

   1) For adherent cells: One day before transfection, seed 1-2x10^5 cells in 500 µL of growth medium without antibiotics per well to make a 70-80% confluency at the time of transfection.

   2) For suspension cells: On the day of transfection just before preparing the complexes, seed 5-10x10^5 cells in 500 µL of growth medium without antibiotics per well.

2. **Preparation before transfection：**

   1) DNA complexes preparation: Dilute 0.5 µg DNA in 25 µL of serum-free medium (such as Opti-MEM) and mix gently. Then add 1 µL of Lipo3K-A Reagent and gently mix to make the DNA complexes.

*Note: This step is for DNA transfect. For siRNA or mRNA transfection, it does not need to add Lipo3K-A Reagent.

   2) Lipo3K diluent preparation: Dilute 1.5 µL Lipo3K-B Reagent in 25 µL of serum-free medium (such as Opti-MEM) and mix gently to make the Lipo3K dilution. Incubate for 5 min at room temperature.

*Note: Lipo3K should be mixed gently before use, do not vortex.

   3) DNA-Lipo3K complexes preparation: After incubation, mix the DNA complexes and Lipo3K diluent gently. Incubate for 20 min at room temperature (solution may appear cloudy). DNA-Lipo3K complexes are stable for 6 h at room temperature.

3. **Cell transfection:** Add the DNA-Lipo3K complexes to prepared cells. Mix gently by gently shaking the plate back and forth. Incubate cells in an incubator for 18-48 h. medium can be changed after 4-6 h.

4. **Detection:** After 24-48 h of incubation, the transfection effect can be detected in appropriate ways, such as fluorescence detection, Western blot, RT-qPCR, ELISA, and reporter gene experiments. Alternatively, a suitable screening antibiotic (e.g., G418) can be added for stable transfection.

5. **Table 1:** The following table shows the recommended dosage for each component for different cell plate transfection experiments.

Culture vessel	Medium		DNA transfection			siRNA transfection	
	Plating medium volume	Dilution medium volume	DNA	Lipo3K-A	Lipo3K-B	siRNA	Lipo3K-B
96-well	100 µL	2x5 µL	0.1 µg	0.2 µL	0.3 µL	3.0 pmol	0.3 µL
48-well	250 µL	2x12.5 µL	0.25 µg	0.5 µL	0.75 µL	7.5 pmol	0.75 µL
24-well	500 µL	2x25 µL	0.5 µg	1 µL	1.5 µL	15 pmol	1.5 µL
12-well	1 mL	2x50 µL	1.0 µg	2 µL	3 µL	30 pmol	3.0 µL
6-well	2 mL	2x125 µL	2.5 µg	5 µL	7.5 µL	75 pmol	7.5 µL
6 cm dish	5 mL	2x250 µL	5.5-11 µg	11-22 µL	16.5 µL	166 pmol	17 µL
10 cm dish	10 mL	2x500 µL	14-28 µg	28-56 µL	43 µL	434 pmol	43 µL

*Note: The dosage in this table is for reference only and can be optimized according to the cell type.

Note

1. The type and viability of the cells have a significant impact on transfection efficiency. Transfection is generally recommended with cells that are in the logarithmic growth phase and have high viability. For adherent cells, it is necessary to ensure that transfection is performed 12-24 h after adherence, otherwise the cells will easily detach. For suspension cells, it is recommended to change the medium ideally 4 h before transfection.
2. The size, quality, and amount of transfection plasmid are also critical to transfection efficiency. High transfection efficiency can be achieved with high-purity plasmids. The plasmid should be free of protein, RNA, and other chemicals, and its $OD_{260/280}$ value should be in the range of 1.8-1.9.
3. It is also necessary to pay attention to the amount of transfection reagent used during transfection, as excessive transfection reagent may also reduce transfection efficiency due to excessive cytotoxicity.
4. This transfection reagent can be used for transfection with a serum-containing medium, but the preparation of DNA-Lipo3K complexes must be in a serum-free medium because serum affects the formation of complexes.
5. The lid of this transfection reagent needs to be closed immediately after use. Because prolonged exposure to air will cause oxidation of lipids and reduce transfection efficiency.
6. This transfection reagent should not be frozen, vortexed, or centrifuged, and should be mixed gently before use.
7. It is not recommended to use antibiotics for transfection.
8. Generally, after 24-48 h of transfection, the target gene can be expressed in cells, and different experiments can be used to detect the expression of the target gene.
9. For your safety and health, please wear lab coats and gloves during the experiment.
10. For research use only. Not to be used in clinical diagnostic or clinical trials.

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