Lipo2K Transfection Reagent

Introduction

Lipo2K 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 some difficult-to-transfect cells. The transfection reagent is used in the same way as the commonly used Lipofectamine 2000 Reagent, and the transfection efficiency of this transfection reagent has been tested to be comparable to or slightly higher than the Lipofectamine® 2000 Reagent.

This transfection reagent has the advantages of high transfection efficiency, simple operation, good reproducibility and no obvious cytotoxicity, and is suitable for transfection of adherent cells and suspension cells. 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	K2704-0.75 mL	K2704-1.5 mL
Lipo2K Transfection Reagent	0.75 mL	1.5 mL
Store the reagent 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 4-8x10^5 cells in 500 µL of growth medium without antibiotics per well.

2. **Preparation before transfection：**

   1) DNA complexes preparation: Dilute 1 μg DNA in 50 μL of serum-free medium (such as Opti-MEM) and mix gently to make the DNA complexes.

   2) Lipo2K diluent preparation: Dilute 2 μL Lipo2K in 50 μL of serum-free medium (such as Opti-MEM) and mix gently to make the Lipo2K dilution. Incubate for 5 min at room temperature.

*Note: Lipo2K should be mixed gently before use, do not vortex.

   3) DNA-Lipo2K complexes preparation: After incubation, mix the DNA complexes and Lipo2K diluent gently. Incubate for 20 min at room temperature (solution may appear cloudy). DNA-Lipo2K complexes are stable for 6 h at room temperature.

3. **Cell transfection:** Add the DNA-Lipo2K 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	Lipo2K	siRNA	Lipo2K
96-well	100 μL	2x25 μL	0.2 μg	0.5 μL	5 pmol	0.25 μL
24-well	500 μL	2x50 μL	0.8 μg	2.0 μL	20 pmol	1.0 μL
12-well	1 mL	2x100 μL	1.6 μg	4.0 μL	40 pmol	2.0 μL
6-well	2 mL	2x250 μL	4.0 μg	10 μL	100 pmol	5.0 μL
6 cm dish	5 mL	2x0.5 mL	8.0 μg	20 μL	200 pmol	10 μL
10 cm dish	15 mL	2x1.5 mL	24 μg	60 μL	600 pmol	30 μL

*Note: The dosage in this table is for reference only and can be optimized according to the cell type. For most cells, a DNA (μg): Lipo2K (μL) ratio of 1:2 to 1:3 can achieve better transfection efficiency and lower cytotoxicity. In addition, the ratio of DNA (μg): Lipo2K (μL) can be optimized from 1:0.5 to 1:5 for optimal transfection efficiency.

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-Lipo2K complexes must be in a serum-free medium because serum affects the formation of complexes.
5. After 4-6 h of transfection, it is necessary to change to the serum-containing medium.
6. 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.
7. This transfection reagent should not be frozen, vortexed, or centrifuged, and should be mixed gently before use.
8. It is not recommended to use antibiotics for transfection.
9. 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.
10. For your safety and health, please wear lab coats and gloves during the experiment.
11. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com