

# ? Ymf Yb' @dc 新型转染试剂

## Keygen Lipo Transfection Reagent

说明书修订日期: 2019.05.21

Cat Number: KGD031/ KGD032/ KGD033/ KGD034

Store at 2-8 °C for 12 months

For Research Use Only (科研专用)

**Table 1. Product Package and Storage**

组分名称	KGD031	KGD032	KGD033	KGD034
Keygen Lipo 新型转染试剂	0.3 mL	0.5 mL	1.0 mL	2.0 mL

## Introduction

Keygen Lipo Transfection Reagent is a lipid-based transfection reagent that forms a complex with DNA or RNA, and transports the complex into a variety of adherent and suspension cell lines. Keygen Lipo Transfection Reagent has been tested to work the same efficiency as Lipo 2000 Reagent, and used for the transfection of both DNA and RNA into eukaryotic cells even in the presence of serum.

## Feature

- Superior transfection efficiency for a broad range of cell lines.
- Does not require removal of serum or culture medium.
- Does not require washing or changing of medium after transfection.
- Low cytotoxicity.

## Protocols

### DNA Transfection

Use the following procedure to transfect DNA into mammalian cells in a 24-well format. For other formats, see Scaling Up or Down Transfections. All amounts and volumes are given on a per well basis. Prepare complexes using a DNA ( $\mu\text{g}$ ) to Keygen Lipo ( $\mu\text{l}$ ) ratio of 1:2 to 1:3 for most cell lines. Transfect cells at high cell density for high efficiency, high expression levels, and to minimize cytotoxicity. Optimization may be necessary (see Optimizing DNA Transfection).

1. **Adherent cells:** One day before transfection, plate  $0.5\text{-}2 \times 10^5$  cells in 500  $\mu\text{l}$  of growth medium without antibiotics so that cells will be 70-90% confluent at the time of transfection. **Suspension cells:** Just prior to preparing complexes, plate  $4\text{-}8 \times 10^5$  cells in 500  $\mu\text{l}$  of growth medium without antibiotics.
2. For each transfection sample, prepare complexes as follows:
  - a. Dilute DNA in 50  $\mu\text{l}$  of Medium without serum. Mix gently.
  - b. Mix Keygen Lipo gently before use, then dilute the appropriate amount in 50  $\mu\text{l}$  of Medium without serum. Incubate for 5 minutes at room temperature. **Note:** Proceed to Step c within 25 minutes.
  - c. After the 5 minute incubation, combine the diluted DNA with diluted Keygen Lipo (total volume = 100  $\mu\text{l}$ ). Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy). **Note:** Complexes are stable for 6 hours at room temperature.
3. Add the 100  $\mu\text{l}$  of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.
4. Incubate cells at 37°C in a CO<sub>2</sub> incubator for 18-48 hours prior to testing for transgene expression. Medium may be changed after 4-6 hours.
5. **For stable cell lines:** Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours after transfection. Add selective medium (if desired) the following day.

## Optimizing DNA Transfection

To obtain the highest transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density as well as DNA and Keygen Lipo concentrations. Make sure that cells are greater than 90% confluent and vary DNA ( $\mu\text{g}$ ): Keygen Lipo ( $\mu\text{l}$ ) ratios from 1:0.5 to 1:5.

## RNA Transfection

Use the following procedure to transfect RNA into mammalian cells in a 24-well format. For other formats, see Scaling Up or Down Transfections. All amounts and volumes are given on a per well basis. Use this procedure as a starting point; optimize transfections as described in Optimizing RNA Transfection.

1. One day before transfection, plate cells in 500  $\mu\text{l}$  of growth medium without antibiotics such that they will be 30-50% confluent at the time of transfection. **Note:** Transfecting cells at a lower density allows a longer interval between transfection and assay time, and minimizes the loss of cell viability due to cell overgrowth.
2. For each transfection sample, prepare oligomer- Keygen Lipo complexes as follows:
  - a. Dilute 20 pmol RNAi or siRNA oligomer in 50  $\mu\text{l}$  Medium without serum (final concentration of RNA when added to the cells is 33 nM). Mix gently.
  - b. Mix Keygen Lipo gently before use, then dilute 1  $\mu\text{l}$  in 50  $\mu\text{l}$  Medium without serum. Mix gently and incubate for 5 minutes at room temperature. **Note:** Proceed to Step c within 25 minutes.
  - c. After the 5-minute incubation, combine the diluted oligomer with the diluted Keygen Lipo. Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy).
3. Add the oligomer- Keygen Lipo complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth. Incubate the cells at 37°C in a CO<sub>2</sub> incubator for 24-96 hours until you are ready to assay for gene knockdown. Medium may be changed after 4-6 hours.

## Optimizing RNA Transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying RNA and Keygen Lipo concentrations. Test 10-50 pmol RNA and 0.5-1.5  $\mu\text{l}$  Keygen Lipo for 24- well format. Depending on the nature of the target gene, transfecting cells at higher densities may also be considered when optimizing conditions.

## Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Keygen Lipo, nucleic acid, cells, and medium used in proportion to the relative surface area, as shown in the table. With automated, high-throughput systems, a complexing volume of 50  $\mu\text{l}$  is recommended for transfections in 96-well plates.

**Note:** You may perform rapid 96-well plate transfections by plating cells directly into the transfection mix. Prepare complexes in the plate and directly add cells at twice the cell density as in the basic protocol in a 100  $\mu\text{l}$  volume. Cells will adhere as usual in the presence of complexes.

Culture vessel	Surface area (cm <sup>2</sup> )	Plating medium volume	Dilution medium volume	DNA transfection		RNA transfection	
				DNA	Keygen Lipo	RNA	Keygen Lipo
96-well	0.3	100 $\mu\text{l}$	2 $\times$ 25 $\mu\text{l}$	0.2 $\mu\text{g}$	0.5 $\mu\text{l}$	5 pmol	0.25 $\mu\text{l}$
24-well	2	500 $\mu\text{l}$	2 $\times$ 50 $\mu\text{l}$	0.8 $\mu\text{g}$	2.0 $\mu\text{l}$	20 pmol	1.0 $\mu\text{l}$
12-well	4	1 ml	2 $\times$ 100 $\mu\text{l}$	1.6 $\mu\text{g}$	4.0 $\mu\text{l}$	40 pmol	2.0 $\mu\text{l}$
6-well	10	2 ml	2 $\times$ 250 $\mu\text{l}$	4.0 $\mu\text{g}$	10 $\mu\text{l}$	100 pmol	5 $\mu\text{l}$
6-cm dish	20	5 ml	2 $\times$ 500 $\mu\text{l}$	8.0 $\mu\text{g}$	20 $\mu\text{l}$	200 pmol	10 $\mu\text{l}$
10-cm dish	60	15 ml	2 $\times$ 1.5 ml	24 $\mu\text{g}$	60 $\mu\text{l}$	600 pmol	30 $\mu\text{l}$