HB221024

# Hieff Trans™ Liposomal Transfection Reagent

## Product Information

| Product Name | Cat# | Size |
|---|---|---|
| Hieff Trans™ Liposomal Transfection Reagent | 40802ES02 | 0.5 mL |
| | 40802ES03 | 1 mL |
| | 40802ES08 | 5×1 mL |

## Product Description

Hieff Trans™ Liposomal Transfection Reagent is a versatile liposome transfection reagent, suitable for DNA, RNA and oligonucleotide transfection, with high transfection efficiency for most eukaryotic cells. Its unique formula allows it to be added directly to the medium, and the presence of serum does not affect transfection efficiency, which reduces the damage to cells caused by serum removal. There is no need to remove the nucleic acid-reagent complex or replace with fresh medium after transfection, and it can also be removed after 4-6 hours.

This product is supplied in sterile liquid form. Usually, for 24-well plate transfection, about 1.5 μL each time, 1 mL of reagent can do about 660 transfections; for 6-well plate, about 6 μL each time, 1 mL of reagent can do about 160 transfections.

## Shipping and Storage

The product is shipped with ice packs and can be stored at 2-8ºC for one year. Do not freeze!

## Cautions

1) Hieff Trans™ Liposomal Transfection Reagent requires a high cell plating density, preferably 60%-80%, which helps to reduce the impact of cationic liposome cytotoxicity; if the gene you are studying requires a long time, for example, cell cycle-related genes or cell surface proteins, it is best to choose transfection reagents with lower cell plating density requirements, and liposomal nucleic acid transfection reagents are not suitable.

2) Hieff Trans™ Liposomal Transfection Reagent can be used for transfection with serum medium, and it is not necessary to change the medium before and after transfection. However, preparation of transfection complexes requires dilution of DNA and transfection reagents in serum-free medium because serum can affect complex formation. In addition, to test the compatibility of the serum-free medium used with the liposomal nucleic acid transfection reagent, CD293, SFMII, VP-SFM are known to be incompatible.

3) Antibiotics should not be added to the medium during transfection.

4) The use of high-purity DNA or RNA helps to obtain higher transfection efficiency, and endotoxin in plasmids is the enemy of transfection.

5) Cationic liposomes should be stored at 4 degrees, and be careful not to open the lid repeatedly for a long time, because it may cause liposome oxidation and affect the transfection efficiency.

6) The DNA concentration and the amount of transfection reagent should be optimized for maximum transfection efficiency for initial use. The ratio of DNA and transfection reagent is usually recommended to be 1:2-1:3, such as seeding 0.5-2×$10^5$ cells in a 24-well plate, using 0.5 μg DNA and 1-1.5 μL of transfection reagent. Optimize the transfection efficiency by adjusting the ratio of DNA/Reagent to ensure that the cell density is greater than 90%, and the ratio of DNA (μg): Reagent(μL) is 1:0.5-1:5.

7) For research use only!

**Instructions** (Take the 24-well plate as an example, please refer to Table 1 for the loading volume of other culture plates)

【Note】 The amount of transfection reagent used is affected by the cell type and other experimental conditions. It is recommended to set a gradient to optimize the optimal amount of use for the first time.

**Adherent cells**: One day before transfection (20-24 hours), trypsinize cells and count, and cells were plated (without antibiotics) to 0.5-2 × $10^5$ cells/well.

**Suspension cells**: On the day of transfection, before preparing DNA complexes, plate cells in 24-well plates at 4-8 × $10^5$ cells per 500 μL of growth medium (without antibiotics).

1. Prepare the DNA-Reagent Complex as follows:

1) For each well of cells, dilute 0.5 μg of DNA with 50 μL of serum-free medium (such as OPTI-MEM I medium). Mix well gently.

2) For each well of cells, dilute 0.6-2.5 μL of transfection reagent with 50 μL of serum-free medium (such as OPTI-MEM I medium). Dilute transfection reagent and incubate at room temperature for 5 mins (mix with the diluted DNA within 30 mins, if the incubation time is too long, the activity will be reduced)

【Note】 If DMEM is used as a diluent for liposomal nucleic acid transfection reagent, it must be mixed with the diluted DNA within 5 mins. Even if the transfection reagent is diluted with OPTI-MEM I, cells can be cultured with DMEM.

2. Mix the diluted DNA and the diluted transfection reagent (total volume 100 μL), mix gently, and incubate at room temperature (15-25°C) for 20 mins to allow the formation of DNA-Reagent complexes. The solution may be cloudy at this point, but it will not affect transfection.

【Note】 DNA-liposome complexes are stable at room temperature for at least 5 h.

3. Add 100 μL of DNA-Reagent complex directly to each well of the cell culture plate, shake the plate, and mix gently.

【Note】 If transfecting under serum-free conditions, use serum-containing normal growth medium for cell plating. Growth medium was removed before complex addition and replaced with 500 μL of serum-free medium.

4. Incubate at 37°C in a 5% $CO_2$ incubator for 24-48 h until transgene expression analysis without removing complexes or changing medium. However, it may be necessary to change the growth medium after 4-6 h without reducing transfection activity.

**Stably transfected cell line:** Selection medium was added 48 h after transfection.

**Suspension cell line:** After the DNA-Reagent complex was added to the cells, PMA and/or PHA could be added 4 h later if desired. For Jurkat cells, final concentrations of PHA and PMA were 1 μg/mL and 50 ng/mL, respectively, which increased CMV promoter activity and gene expression. For K562 cells, the addition of PMA alone was sufficient to increase promoter activity.

**Adjustment of transfection system**

For different cell culture plates, the amount of transfection reagent, DNA, cells and medium will be different, please refer to the following table (Table 1) for details. For 96-well plate culture, there is no need to plate cells one day in advance, and the complex can be prepared directly in the plate, and then the cell suspension can be added to the complex, which further reduces the transfection time. This modified procedure has been tested with 293-H, 293-F, COS-7L and CHO cells and is slightly less active than the traditional method. Fast steps and efficient transfection of protein-expressing cell lines make liposomal nucleic acid transfection reagents ideal for high-throughput transfection in 96-well plates, such as cDNA library screening and transient protein expression.

**Table 1 Amounts of transfection reagent, nucleic acid, cells and medium for different culture vessels**

| Culture vessel | Surf. area per well [1] | Shared reagents | | DNA transfection | | RNAi transfection | |
|---|---|---|---|---|---|---|---|
| | | Vol. of plating medium | Vol. of dilution medium [2] | DNA | transfection reagent | RNA | transfection reagent |
| 96-well | 0.3 $cm^2$ | 100 μL | 2×25 μL | 0.1 μg | 0.2-0.5 μL | 5 pmol | 0.25 μL |
| 24-well | 2 $cm^2$ | 500 μL | 2×50 μL | 0.5 μg | 0.6-2.5 μL | 20 pmol | 1.0 μL |
| 12-well | 4 $cm^2$ | 1 mL | 2×100 μL | 1 μg | 2-4.5 μL | 40 pmol | 2.0 μL |
| 6-well | 10 $cm^2$ | 2 mL | 2×250 μL | 2-4 μg | 5-10 μL | 100 pmol | 5 μL |
| 60-mm | 20 $cm^2$ | 5 mL | 2×0.5 mL | 4-8 μg | 10-20 μL | 200 pmol | 10 μL |
| 10-cm | 60 $cm^2$ | 15 mL | 2×1.5 mL | 12-24 μg | 30-60 μL | 600 pmol | 30 μL |

1 The surface area of cell culture plates provided by different manufacturers may vary;

2 Volume of medium used to dilute DNA or RNAi.

【Note】 The usage amount in this table is for reference only, and the specific usage amount needs to be optimized according to the cell type and other experimental conditions. DNA (μg):Reagent (μL) ratio was kept at 1:0.5-1:5 when use.