Ver. HB230117

Hieff Trans™ *in vitro* siRNA/miRNA Transfection Reagent

Product description

Hieff Trans™ *in vitro* siRNA/miRNA Transfection Reagent is a PEI cationic, amphiphilic transfection reagent developed for transfection of siRNA into mammalian cells. Suitable for transfection of siRNA and miRNA. This transfection reagent encapsulates siRNA or miRNA to form a cationic complex, which interacts with negatively charged proteoglycans on the cell surface and adsorbs on the cell surface, enters the cell through endocytosis, and forms an inclusion body. The transfection reagent acts as a proton sponge, which can absorb H^+ in the lysosome, and the continuous influx of protons causes the complex to swell and rupture, thereby releasing the exogenous siRNA or miRNA into the cytoplasm.

This product can achieve over 90% expression efficiency of 1 nM siRNA in a wide range of cell lines, avoiding off-target effects. Suitable for transfection of a variety of cells, including Hela, MCF-7, HepG2, CHO and other adherent cells; and difficult to transfect suspension cell lines, such as K562 or THP-1 cells, can achieve 80% silencing efficiency; Including some primary cells, primary human fibroblasts and primary human hepatocytes, etc., the silencing efficiency of 80% can be achieved.

Components

Components No.	Name	40806ES01	40821ES02	40821ES03
40806	Hieff Trans™ *in vitro* siRNA/miRNA Transfection Reagent	0.1 mL	0.5 mL	1 mL

Specifications

Form	Liquid
Serum Compatible	Yes
Cell Type	Established Cell Lines
Sample Type	siRNA/miRNA
Transfection Technique	PEI-Based Transfection

Storage

The product should be stored at 2-8°C for one year. Do not freeze!

Instructions

1. Transfection of adherent cells (take 24-well plate and transfection of 1 nM siRNA as an example, please refer to Table 1 for the loading volume of other culture plates)

1.1 In order to improve the transfection efficiency, it is recommended to inoculate the cells one day before the transfection. The density of the inoculated cells is recommended to be about 30%-50%. It is recommended to set a gradient to optimize the optimal usage amount when using it for the first time.

1.2 Prepare the complex of siRNA-PEI or miRNA-PEI sub-nucleic acid transfection reagent according to the following

system:

1.2.1 For each well of cells, dilute 8.4 ng of siRNA (0.6 pmoles) with 100 μL of serum-free medium (such as OPTI-MEM I medium) and mix well.

1.2.2 Immediately add 2 μL of transfection reagent to 100 μL of siRNA, and mix gently.

1.2.3 Incubate at room temperature for 10 min to form the siRNA-PEI cationic nucleic acid transfection reagent complex.

【Note】 The incubation time should not exceed 30 min

1.2.4 During complex formation, remove cell growth medium and add 500 μL of fresh prewarmed complete medium to each well.

1.2.5 Add 100 μL of siRNA-PEI cationic nucleic acid transfection reagent complex directly into the cells, shake the culture plate, and mix gently. The final volume is 600 μL and the final siRNA concentration is 1 nM.

1.2.6 Cultivate at 37°C, 5% CO_2 incubator until the target gene is expressed. It is suggested that the general mRNA expression level is usually 24-72 h, and the protein expression level is 48-96 h.

Table 1. When the final concentration of siRNA is 1 nM, the reference value of the dosage of transfection reagent and medium

Culture vessel	Surf. area per well[1] (cm^2)	siRNA (pmoles)	Amount of siRNA per well(ng)	Transfection reagent(μL)	Vol. of dilution medium[2](μL)	Vol. of plating medium
96-well	0.3	0.17	2.4	0.7-0.8	50	175 μL
24-well	1.9	0.6	8.6	1-3	100	600 μL
12-well	3.8	1.2	17	2-6	200	1.2 mL
6-well	10	2.2	31	4-12	200	2.2 mL
Flask 25cm^2	21	4.4	62	10-20	400	4.4 mL
Flask 75cm^2	58	10.5	147	30-50	500	10.5 mL

Note: 1) The surface area of cell culture plates provided by different manufacturers may vary.

2) Volume of medium used to dilute DNA.

Table 2. When the final concentration of siRNA is 10 - 50 nM, the reference value of the dosage of transfection reagent and medium

Culture vessel	Surf. area per well[1] (cm^2)	Transfection reagent (μL)	Vol. of dilution medium[2] (μL)	Vol. of plating medium
96-well	0.3	0.5-1.5	50	175 μL
24-well	1.9	2-4	100	600 μL
12-well	3.8	4-6	200	1.2 mL
6-well	10	8-16	200	2.2 mL
Flask 25cm^2	21	15-25	400	4.4 mL

Note：1) The surface area of cell culture plates provided by different manufacturers may vary.

2) Volume of medium used to dilute complexation.

2. Suspension cell transfection (take 24-well plate and transfection of 5 nM siRNA as an example, please refer to Table 4 for the loading volume of other culture plates)

2.1 To optimize transfection conditions for suspension cells, the volume of medium for suspension cells needs to be reduced compared to adherent cells. Depending on the dish size and the volume of complete medium, the recommended number of cells to seed in suspension is shown in Table 3 below.

Table 3. On the day of transfection, according to the size of the culture dish, the reference value of the number of cells inoculated in suspension

Culture vessel	Surf. area per well[1)] (cm^2)	Vol. of cell (μL)	Cell number
384-well	0.1	25	5×10^3-1×10^4
96-well	0.3	50	1×10^4-2×10^4
24-well	1.9	200	1×10^5-2×10^5
12-well	3.8	500	2×10^5-4×10^5
6-well	10	1000	5×10^5-2×10^6
Flask 25cm^2	21	2000	2×10^6-5×10^6

Note：1) The surface area of cell culture plates provided by different manufacturers may vary.

2.2 Prepare the siRNA-PEI cationic nucleic acid transfection reagent complex according to the following system:

2.2.1 For each well of cells, dilute 21 ng siRNA (1.5 pmols) with 100 μL of serum-free medium (such as OPTI-MEM I medium) and mix well.

2.2.2 Add 4 μL of transfection reagent to 100 μL of siRNA, vortex immediately for 10 seconds, and mix gently.

2.2.3 Incubate for 15 min at room temperature to form the siRNA-PEI cationic nucleic acid transfection reagent complex.

【Note】 The incubation time should not exceed 30 min

2.2.4 Add 100 μL of siRNA-PEI cationic nucleic acid transfection reagent complex to 200 μL of cell suspension in each well, and mix gently. The final volume is 300 μL, and the final siRNA concentration is 5 nM.

2.2.5 After culturing for 4-6 hours in a 37°C, 5% CO_2 incubator, add 700 μL of complete medium, and gently shake the culture plate to mix.

2.2.6 Continue to incubate in the incubator until the target gene is expressed. It is suggested that the general mRNA expression level is usually 24-72 h, and the protein expression level is 48-96 h.

【Note】 In order to optimize endogenous gene silencing, it is recommended to select the siRNA concentration between 5 nM and 20 nM. The volume of transfection reagent should be adjusted according to the siRNA concentration and the size of the petri dish, see Table 4 below.

Table 4. Reference values in suspension cells at 5 nM siRNA concentration

Culture vessel	siRNA (pmoles)	Amount of siRNA per well(ng)	Transfection reagent(μL)	Vol. of dilution medium [1)](μL)	Vol. of cell	Vol. of additional medium[2)]
384-well	0.25	3.75	1±0.5	25	25 μL	0 μL
96-well	0.5	7.5	2±1	50	50 μL	100 μL
24-well	1.5	21	3±2	100	200 μL	700 μL
12-well	3.5	49	6±4	200	500 μL	1 mL
6-well	6	84	10±8	200	1 mL	2 mL

Flask 25cm^2	12	168	15±10	400	2 mL	4 mL

Note: 1) Volume of medium used to dilute complexation.

2) Volume of additional medium added 4-6 h after transfection.

Table 5. Reference values of siRNA concentration and transfection reagent dosage in suspension cells

Culture vessel	Final concentration of siRNA /well(nM)	Transfection reagent/well(μL)
96-well	1 ~ 20	1 ± 0.5
24-well		2 ± 1
12-well		3 ± 2
6-well		10 ± 8
96-well	20 ~ 50	1.5 ± 0.5
24-well		3 ± 1
12-well		5 ± 2
6-well		15 ± 8

3. miRNA transfection procedure

This transfection reagent is also suitable for transfecting miRNA. For the specific operation of transfecting adherent cells and suspension cells, please refer to the siRNA operation process.

Notes

1. Use RNase-free and non-pyrogenic materials throughout the experiment, such as centrifuge tubes, pipette tips, and buffers.

2. Before transfection, make sure that the siRNA has been purified by PAGE and desalted. High-purity siRNA or miRNA helps to obtain higher transfection efficiency.

3. The PEI cationic transfection reagent should be stored at 2-8°C, and care should be taken to avoid repeatedly opening the lid for a long time, otherwise it may cause PEI to volatilize and reduce the transfection efficiency.

4. One day before transfection, make sure that the density of adherent cells is around 30%-50%. For some small cells and slow-growing cells, the density can be appropriately increased by 2 times.

5. Before transfection, ensure that siRNA/miRNA gene silencing expression will not affect cell viability.

6. This product is only suitable for *in vitro* transfection and can not be used for *in vivo* transfection.

7. For research use only!