

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

## Product Information

Product Name	Cat#	Size
	40806ES01	0.1 mL
Hieff Trans™ siRNA/miRNA <i>in Vitro</i> Transfection Reagent	40806ES03	0.5 mL
	40806ES08	1.0 mL

## Product Description

Hieff Trans™ siRNA/miRNA *in Vitro* Transfection Reagent is a non-liposomal 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<sup>+</sup> 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.

## Shipping and Storage

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

## Cautions

- 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 4 °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!

## 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)**

### seeding adherent cells

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 cationic complex of siRNA-PEI or miRNA-PEI sub-nucleic acid transfection reagent according to the following system:

- 1) For each well of cells, dilute 8.4 ng of siRNA (0.6 pmoles) with 100  $\mu$ L of serum-free medium (such as OPTI-MEM I medium) and mix well.
- 2) Immediately add 2  $\mu$ L of transfection reagent to 100  $\mu$ L of siRNA, vortex for 10 seconds, and mix well.
- 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 30min
- 4) During complex formation, remove cell growth medium and add 500  $\mu$ L of fresh prewarmed complete medium to each well.
- 5) Add 100  $\mu$ L 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  $\mu$ L and the final siRNA concentration is 1 nM.
- 6) Cultivate at 37°C, 5% CO<sub>2</sub> 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.

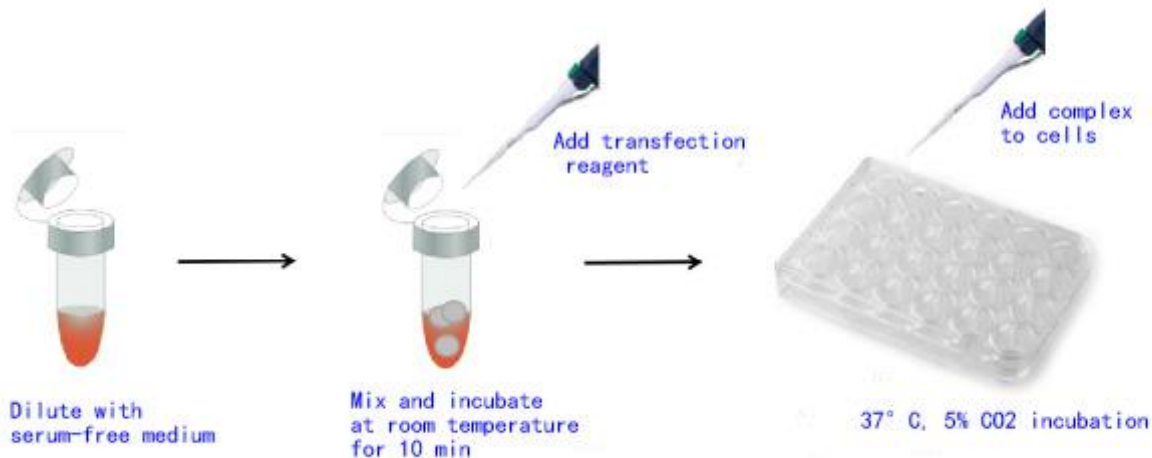


Figure 1. Procedure for transfection of adherent cells

【Note】 The transfection efficiency of siRNA will be affected by factors such as target gene, cell type, siRNA titer, half-life of target mRNA and turnover rate of target protein. The recommended siRNA concentration is between 1 nM and 10 nM. The volume of transfection reagent should be adjusted according to the siRNA concentration and the size of the petri dish, see Table 1 below.

**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 <sup>1</sup> (cm <sup>2</sup> )	siRNA (pmoles)	Amount of siRNA per well(ng)	Transfection reagent( $\mu$ L)	Vol. of dilution medium <sup>2</sup> ( $\mu$ L)	Vol. of plating medium
96-well	0.3	0.17	2.4	0.7-0.8	50	175 $\mu$ L
24-well	1.9	0.6	8.6	1-3	100	600 $\mu$ L
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 <sup>2</sup>	21	4.4	62	10-20	400	4.4 mL
Flask 75cm <sup>2</sup>	58	10.5	147	30-50	500	10.5 mL

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

2. Volume of medium used to dilute complexation.

**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 <sup>1</sup> (cm <sup>2</sup> )	Transfection reagent(μL)	Vol. of dilution medium <sup>2</sup> (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 <sup>2</sup>	21	15-25	400	4.4 mL

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)**

**seeded cells**

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 <sup>1</sup> (cm <sup>2</sup> )	Vol. of cell (μL)	Cell number
384-well	0.1	25	5×10 <sup>3</sup> -1×10 <sup>4</sup>
96-well	0.3	50	1×10 <sup>4</sup> -2×10 <sup>4</sup>
24-well	1.9	200	1×10 <sup>5</sup> -2×10 <sup>5</sup>
12-well	3.8	500	2×10 <sup>5</sup> -4×10 <sup>5</sup>
6-well	10	1000	5×10 <sup>5</sup> -2×10 <sup>6</sup>
Flask 25cm <sup>2</sup>	21	2000	2×10 <sup>6</sup> -5×10 <sup>6</sup>

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:

- 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) Add 4 μL of transfection reagent to 100 μL of siRNA, vortex immediately for 10 seconds, and mix well.
- 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

- 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.
- 5) After culturing for 4-6 hours in a 37 °C, 5% CO<sub>2</sub> incubator, add 700 μL of complete medium, and gently shake the culture plate to mix.
- 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( $\mu$ L)	Vol. of dilution medium <sup>1</sup> ( $\mu$ L)	Vol. of cell	Vol. of additional medium <sup>2</sup>
384-well	0.25	3.75	1 $\pm$ 0.5	25	25 $\mu$ L	0 $\mu$ L
96-well	0.5	7.5	2 $\pm$ 1	50	50 $\mu$ L	100 $\mu$ L
24-well	1.5	21	3 $\pm$ 2	100	200 $\mu$ L	700 $\mu$ L
12-well	3.5	49	6 $\pm$ 4	200	500 $\mu$ L	1 mL
6-well	6	84	10 $\pm$ 8	200	1 mL	2 mL
Flask 25cm <sup>2</sup>	12	168	15 $\pm$ 10	400	2 mL	4 mL

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( $\mu$ L)
96-well	1 ~ 20	1 $\pm$ 0.5
24-well		2 $\pm$ 1
12-well		3 $\pm$ 2
6-well		10 $\pm$ 8
96-well	20 ~ 50	1.5 $\pm$ 0.5
24-well		3 $\pm$ 1
12-well		5 $\pm$ 2
6-well		15 $\pm$ 8

### 3 miRNA transfection workflow

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.