T-Pro NTR I Transfection Reagent

Store at 2~8°C

(JT97-N001M) 1.0ml

T-Pro Biotechnology

This product is for laboratory research ONLY and not for diagnostic use.

Introduction	T-Pro NTR I Transfection Reagent is a proprietary formulation that could be transfected DNA and RNA into eukaryotic cells and it is a forms of the covalent binding with nucleic acid by cation. In the meanwhile, T-Pro NTR I Transfection Reagent to provide the highly efficiency and low cytotoxicity while transfected. T-Pro NTR I Transfection Reagent can be used in many cells type including HEK293, 293T, 293E, BHK, CHO, COS1, COS7, HeLa, Vero, PC12, L929, NIH 3T3, Human Foreskin Fibroblasts (HFF) primary cells, Bovine Aorta Endothelial cells(BAEC), Sf9, Sf21, etc. Furthermore, T-Pro NTR I Transfection Reagent also has high transfection efficiency when applied in other establishing stable eukaryotic cells. T-Pro NTR I Transfection Reagent is efficient to deliver DNA, RNA, siRNA, oligonucleotides, ribonucleoprotein particles & protein into cells and has attractive power on price. You could make sure to decrease the cost greatly on experiment.
Characteristics	- Low cytotoxicity for most of tumor cell lines and primary cells - Exceptional transfection efficiency on the experiment for a broad range of cell types - Efficient transfection with or without serum - High levels of recombinant protein production - Simple, robust transfection on procedure - Efficient transfection for adhesion cells or suspend cells.
Storage	T-Pro NTR I Transfection Reagent is stable for 2~8°C

Procedure

a)For adhesion cells:

*The number of cells to seed (see Table 1).The cells should be cultured before the transfection in 18 to 24 hours and make sure the density of cells could be reached to 40~90 % at the day of the transfection.Also to change clean culture medium before transfection in 0.5-2.0 hours.

*Use the following procedure to transfect cells in a 24-well format. All amounts and volumes are given on a per well basis. Please scaling up or down volume if need to do a transfection on difference form of well (see Table 4).

1. Please use culture medium with serum in special condition; you could be used serum-free culture medium (without antibiotics) or culture medium with serum lower than 5%. It could improve the expression of recombinant protein or increase the efficienty of transfection.

2. The day before transfection, plate 0.5-2 x 105 cells in 500 µl of growth medium without antibiotics so that cells will be 90-95% confluent at the time of transfection.

*For difference cell types, the optimizational rate for T-Pro NTR I Transfection Reagent (µl) : DNA (µg) should be (2：1 to 4：1).The recommendatory rate is 3:1 in beginnings (See table 2).

Protocol for the gene transfection of mammalian cells

1	Pipet the Serum-free Culture medium (without antibiotics) 30µl/well or HBS buffer (pH7.4 150mM NaCi, 20mM HEPES) in a sterile plastic tube.
2	Add plasmid DNA 1µg/well into Serum-free culture medium and mix by gentle pipetting.
3	Pipet with drop of T-Pro NTR I Transfection Reagent 2~4µl/well over tube with DNA and mix by gentle.
4	Incubate the mixture of DNA and T-Pro NTR I Transfection Reagent at room temperature for 15 minutes.
5	Pipet DNA/ T-Pro NTR I Transfection Reagent complex to cells in each well and mix by gentle and shaking plate.
6	Incubate cells at 37°C in a incubator with 5% CO_2 for 18-48 hrs.

Procedure

b)For suspend cells :

*Cell Seeding: Suspension cells are typically seeded at a density of 1.0~2.0 x 105 (0.5-1.0 x 106) cells/ml of culture on the day of transfection. For optimal transfection conditions with T-Pro NTR I Transfection Reagent, seed the number of cells adapted to the culture vessel format according to Table 3.

*Use the following procedure to transfect cells in a 24-well format. All amounts and volumes are given on a per well basis. Please scaling up or down volume if need to do a transfection on difference form of well (see Table 4).

*For difference cell types, the optimizational rate for T-Pro NTR I Transfection Reagent (μl) : DNA (μg) should be (2：1 to 4：1).The recommendatory rate is 3:1 in beginnings (See table 2).

Protocol for the gene transfection of mammalian cells

1	Pipet the Serum-free Culture medium (without antibiotics) 60μl/well or HBS buffer(pH7.4 150mM NaCi, 20mM HEPES) in a sterile plastic tube.
2	Add plasmid DNA 1μg/well into Serum-free culture medium and mix by gentle pipetting.
3	Pipet with drop of T-Pro NTR I Transfection Reagent 2~4μl/well over tube with DNA and mix by gentle.
4	Incubate the mixture of DNA and T-Pro NTR I Transfection Reagent at room temperature for 15 minutes.
5	Pipet DNA/ T-Pro NTR I Transfection Reagent complex to cells in each well and mix by gentle and shaking plate.
6	Incubate cells at 37°C in a incubator with 5% CO_2 for 18-48 hrs.

Transfection experiments are usually stopped after 24 to 48 hours and gene activity assessed. Cells growing in suspension are collected by centrifugation at 800 x g and then re-suspended in the desired medium or buffer.

Procedure

c)siRNA Transfection:

*Use the following procedure to transfect cells in a 24-well format. All amounts and volumes are given on a per well basis. Please scaling up or down volume if need to do a transfection on difference form of well (see Table 4).

*For difference cell types, the optimizational rate for T-Pro NTR I Transfection Reagent (μl) : siRNA (μg) should be (2：1 to 4：1).The recommendatory rate is 3:1 in beginnings.

Protocol for the gene transfection of mammalian cells

1	Pipet the Serum-free Culture medium (without antibiotics) 30μl/well or HBS buffer (pH7.4 150mM NaCi, 20mM HEPES) in a sterile plastic tube.
2	Add siRNA 5~50 pmol into Serum-free culture medium and mix by gentle pipetting.
3	Pipet with drop of T-Pro NTR I Transfection Reagent 1~5μl/well over tube with DNA and mix by gentle.
4	Incubate the mixture of siRNA and T-Pro NTR I Transfection Reagent at room temperature for 15 minutes.
5	Pipet siRNA/ T-Pro NTR I Transfection Reagent complex to cells in each well and mix by gentle and shaking plate.
6	Incubate cells at 37°C in a incubator with 5% CO_2 for 18-48 hrs.

*The concentration of siRNA should be above the solution of 1μM

Table 1. A Guideline for Seeding Adherent Cells Prior to Transfection in Different Culture Formats.

Culture Dishes	Surface Area (cm2)	Number of Cells to Seed
T175 Flask	175	0.7 – 1.4 x 10^7
T75 Flask	75	3.0 – 6.0 x 10^6
100 mm Dish	58	2.2 – 4.4 x 10^6
60 mm Dish	21	0.9 – 1.8 x 10^6
35 mm Dish	9.6	3.5 – 7.0 x 10^5
6-well Plate	9.6	4.0 – 8.0 x 10^5
12-well Plate	3.5	1.5 – 3.0 x 10^5
24-well Plate	1.9	0.8 – 1.6 x 10^5
48-well Plate	1.0	4.0 – 8.0 x 10^4
96-well Plate	0.3	1.2 – 2.4 x 10^4

Table 2. Recommended Amounts for Different Culture Vessel Formats.

Culture Dish	Culture Volume (ml)	Plasmid DNA (μg)	Diluent Volume (μl)	T-Pro NTR I (μl)
96 well plate	**0.1**	**0.2**	**10**	**0.4~0.8**
24 well plate	**0.5**	**1.0**	**30**	**2~4**
6 well plate	**2.0**	**4.0**	**120**	**8~16**
35 mm dish	**2.0**	**4.0**	**120**	**8~16**
60 mm dish	**5.0**	**10.0**	**300**	**20~40**
100 mm dish	**15.0**	**30.0**	**900**	**60~120**

Table 3. Recommended Number of Suspension Cells to Seed.

Culture Dish	Number of Cells
96-well plate	$2 \times 10^4 - 5 \times 10^4$
48-well plate	$5 \times 10^4 - 1 \times 10^5$
24-well plate	$1 \times 10^5 - 2 \times 10^5$
6-well plate	$2 \times 10^5 - 5 \times 10^5$
35 mm dish	$5 \times 10^5 - 2 \times 10^6$
60 mm dish	$2 \times 10^6 - 5 \times 10^6$
100 mm dish	$5 \times 10^6 - 1 \times 10^7$

Table 4. Scaling Up or Down Transfections

Use the multiplication factor in the following table to scale the volumes for your transfection experiment. The factor is based on the relative surface area of a single well from a 24-well plate.

Culture Vessel	Multiplication Factor	Culture Vessel	Multiplication Factor
96-well plate	0.2X	6-well plate	4X
48-well plate	0.5X	60 mm dish	10X
24-well plate	1X	100 mm dish	30X
12-well plate	2X	T75 flask	40X