T-Pro Transfection Reagent II

Store at 2~8°C

(JT98-T002S) 0.5 ml

(JT98-T002M) 1.0 ml

T-Pro Biotechnology

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

Description	T-Pro Transfection Reagent II is a proprietary formulation for the transfection of DNA and RNA into eukaryotic cells and forms the covalent binding with nucleic acid to provide the highly efficiency and low cyto-toxicity. T-Pro Transfection Reagent II can be used in many cell types including HEK293, 293T, 293E, CHO, COS1, HeLa, NIH 3T3, Sf9 and Sf21. Furthermore, T-Pro Transfection Reagent II also has high transfection efficiency when applied in other eukaryotic cells
Properties	- Low cytotoxicity for most of tumor cell lines and primary cells. - Exceptional transfection efficiency of a broad range of cell types. - Efficient transfection with or without serum. - High levels of recombinant protein production. - Simple, robust transfection procedure.
Storage	T-Pro Transfection Reagent II is stable for 2~8°C

Procedure

For adhesion cells

Note: Use the following procedure to transfect mammalian cells in a 6-well plate format. For other formats, see Scaling Up or Down Transfections. All amounts and volumes are given on a per well basis.

1. One day before transfection, plate 0.5-2 x 10^5 cells in 500 µl of growth medium without antibiotics so that cells will be 70-95% confluent at the time of transfection (table 1).
2. For each transfection sample, prepare complexes as follows T-Pro Transfection Reagent II (µl) : DNA (µg) (3 ： 1~12 ： 1)
   a. Dilute DNA 2.5µl (1µg/µl) in 25 µl of serum free medium or HBS buffer (pH 7.4, 150 mM NaCl, 20mM HEPES) Mix gently.
   b. Mix T-Pro Transfection Reagent III 15µl gently before use, and then dilute the appropriate amount in 50 µl of serum free medium or HBS buffer (pH 7.4, 150 mM NaCl, 20mM HEPES) Mix gently. Incubate for 5 minutes at room temperature.
   Note: Combine diluted T-Pro Transfection Reagent II with diluted DNA within 30 minutes.
   c. After 5 minute incubation, combine the diluted DNA with diluted T-Pro Transfection Reagent II (total volume = 100 µl). Mix gently and incubate for 15 minutes at room temperature (solution may appear cloudy).
3. Add the 100 µl 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_2 incubator for 24-48 hours prior to testing for transgene expression. It is not necessary to change the medium, but medium may be replaced after 6-24 hours.

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

Procedure

For suspend cells

The following protocol is given for transfection in 6-well plate. The protocol can be scalded up or down according to culture volume.

Cell Seeding: Suspension cells are typically seeded the day of the transfection at a density of 1.0~3.0 x 10^5 cells per ml of culture. For optimal transfection conditions with T-Pro Transfection Reagent II, seed the number of cells adapted to the culture vessel format according to Table 3.

1. For each well, dilute 5 µg of DNA into 50 µl of Serum-free Medium with High Glucose. Vortex gently and spin down briefly.
2. For each well, dilute 30 µl of T-Pro Transfection Reagent II reagent into 100 µl of Serum-free Medium with High Glucose. Vortex gently and spin down briefly.
3. Add the 130 µl T-Pro Transfection Reagent II solution immediately to the 55 µl DNA solution all at once.
4. Vortex- mix the solution immediately and spin down briefly to bring drops to the bottom of the tube.
5. Incubate for 15 minutes at room temperature.
6. Add the 200 µl T-Pro Transfection Reagent II / DNA mixture drop-wise onto the serum-containing medium in each well, homogenize the mixture by gently swirling the plate.
7. Incubate at 37 0C and 5% CO_2 in a humidified atmosphere.

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 resuspended in the desired medium or buffer.

Table 2. Recommended Amounts for Different Culture Vessel Formats.

1	**Subculture cells the day before transfection**				
		Adherent cells			Cells in suspension
	Culture dish diameter	35 mm	60 mm	100 mm	-
	Cell number (yielding 60-80% confluency)	1.0–3.0 × 10^5/dish	0.5–1.0 × 10^6/dish	1.5–2.5 × 10^6/dish	1.0 –3.0 × 10^5/ml
	Culture medium volume	1–2 ml	5–6 ml	10–14 ml	2–5 ml
2	**Prepare the T-Pro Transfection Reagent II/DNA mixture**				
2a	**Dilute DNA to a final volume of**	~2.5 μg 25 μl	~5 μg 50 μl	~7.5 μg 75 μl	~5 μg 50 μl
2b	**Dilute T-Pro Transfection Reagent II toa final volume of**	15 μl 50 μl	30 μl 100 μl	45 μl 140 μl	30 μl 100 μl
	Mix the DNA (2a) and T-Pro Transfection Reagent II (2b) solutions and incubate			**10–15 min**	**15–25°C**
3	**Transfect cells**				
	Replace culture medium by fresh medium Containing the T-Pro Transfection Reagent II /DNA mixture			**3~24 h**	37°C
4	**Further culture cells according to the experimental design**				**37°C**

Table 3. Recommended Number of Suspension Cells to Seed.

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