

T-Pro Transfection Reagent III

T-Pro
Biotechnology

Store at
2~8°C

(JT98-T003S) 0.5 ml

(JT98-T003M) 1.0 ml

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

Description	<p>T-Pro Transfection Reagent III 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.</p> <p>T-Pro Transfection Reagent III can be used in many cell types including HEK293, 293T, 293E, CHO, COS1, HeLa, NIH 3T3, Sf9 and Sf21. Furthermore, T-Pro Transfection Reagent III also has high transfection efficiency when applied in other eukaryotic cells.</p>
Properties	<ul style="list-style-type: none">- 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 III 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 $0.5-2 \times 10^5$ cells in 500 μ l 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 III (μ l) : DNA (μ g) (3 : 1~12 : 1)
 - a. Dilute DNA 2.5 μ l (1 μ g/ μ l) in 25 μ l of serum free medium or HBS buffer (pH 7.4, 150 mM NaCl, 20mM HEPES) Mix gently.
 - b. Mix T-Pro Transfection Reagent III 15 μ l gently before use, and then dilute the appropriate amount in 50 μ l 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 III with diluted DNA within 30 minutes.
 - c. After 5 minute incubation, combine the diluted DNA with diluted T-Pro Transfection Reagent III (total volume = 100 μ l). Mix gently and incubate for 15 minutes at room temperature (solution may appear cloudy).
- 3 Add the 100 μ l 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 CO₂ 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 (cm ²)	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 scaled 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 10⁵ cells per ml of culture. For optimal transfection conditions with T-Pro Transfection Reagent III, 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 III 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 III 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 III / DNA mixture drop-wise onto the serum-containing medium in each well, homogenize the mixture by gently swirling the plate.
- 7 Incubate at 37 °C and 5% CO₂ 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 III / 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 III to a 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 III(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 III/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 × 10 ⁴ - 5 × 10 ⁴
48-well plate	5 × 10 ⁴ - 1 × 10 ⁵
24-well plate	1 × 10 ⁵ - 2 × 10 ⁵
6-well plate	2 × 10 ⁵ - 5 × 10 ⁵
35 mm dish	5 × 10 ⁵ - 2 × 10 ⁶
60 mm dish	2 × 10 ⁶ - 5 × 10 ⁶
100 mm dish	5 × 10 ⁶ - 1 × 10 ⁷