# T-Pro Transfection Reagent I

Store at 2~8°C

(JT98-T001S) 0.5 ml

(JT98-T001M) 1.0 ml

T-Pro Biotechnology

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

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| **Description** | T-Pro Transfection Reagent I 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.<br>T-Pro Transfection Reagent I can be used in many cell types including HEK293, 293T, 293E, CHO, COS1, HeLa, NIH 3T3, Sf9 and Sf21. Furthermore, T-Pro Transfection Reagent I also has high transfection efficiency when applied in other eukaryotic cells. |
| **Properties** | - Low cytotoxicity for most of tumor cell lines and primary cells.<br>- Exceptional transfection efficiency of a broad range of cell types.<br>- Efficient transfection with or without serum.<br>- High levels of recombinant protein production.<br>- Simple, robust transfection procedure. |
| **Storage** | T-Pro Transfection Reagent I 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 I (µl) : DNA (µg) (2 ： 1~6 ： 1):
   a. Dilute DNA 4µl (1µg/µl) in 50 µl of serum free medium. Mix gently.
   b. Mix T-Pro Transfection Reagent I 10µl gently before use, and then dilute the appropriate amount in 50 µl of serum free medium. Incubate for 5-10 minutes at room temperature. Note: Combine diluted T-Pro Transfection Reagent I with diluted DNA within 30 minutes.
   c. After 5-10 minute incubation, combine the diluted DNA with diluted T-Pro Transfection Reagent I (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 0.5~1.0 x $10^6$ 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 2 µg of DNA into 100 µl of Serum-free Medium with High Glucose. Vortex gently and spin down briefly.
2. For each well, dilute 10 µl of T-Pro Transfection Reagent I reagent into 100 µl of Serum-free Medium with High Glucose. Vortex gently and spin down briefly.
3. Add the 100 µl T-Pro Transfection Reagent I solution immediately to the 100 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 I / 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.

| Culture Dish | Culture Volume (ml) | Plasmid DNA (ug) | Diluent Volume (ml) | T-Pro Transfection Reagent I (ul) |
|---|---|---|---|---|
| 6 well plate | **1.6** | **2~4** | **0.2** | **5~10** |
| 35 mm dish | **1.6** | **2~4** | **0.2** | **5~10** |
| 60 mm dish | **4.5** | **5~10** | **0.5** | **12.5~25** |
| 100 mm dish | **8** | **7~8** | **1.0** | **17.5~20** |
| T75 flask | **15** | **18~36** | **1.5** | **45~90** |
| 250 ml flask | **50** | **50~100** | **2.5** | **125~250** |

**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 | $5 \times 10^6 - 1 \times 10^7$ |