☐ F90101TH-05: 0.5mL
☐ F90101TH-10: 1.0mL
☐ F90101TH-101: 10x1.0mL

Trans-Hi™ *In Vitro* DNA Transfection Reagent

----- A General Protocol for Transfecting Mammalian Cell

Introduction:

Trans-Hi™ Transfection Reagent is formulated to be a powerful DNA transfection reagent with superior and reproducible transfection and low cytotoxicity. Trans-Hi™ was proven to deliver genes to various established cell lines as well as primary cells.

Important Notes for Transfection:

1. The following general protocol is for DNA transfecting mammalian cells only. Protocols for lentivirus, rAAV or adenovirus production are available for download at www.liposomes.com
2. For better efficiency, choosing a correct protocol is essential. We strongly encourage to use this "General Protocol" first. If it fails to give satisfactory result (e.g., less than 10%) try the "Advanced Protocol" available at www.liposomes.com.
3. For high efficiency and lower toxicity, transfect cells at high density. 70 ~ 80% confluency is highly recommended
4. For efficient transfection and low cytotoxicity conduct transfection in the presence of serum (10%) and antibiotics. High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. However the Trans-Hi™/ DNA complexes must be prepared in serum-free DMEM with High Glucose.

Part I. General Procedures for Transfecting Adherent Cells

Step I. Cell Seeding:

1. One day (18 to 24 hours) prior to transfection plated sufficient number of (approx. 0.5 x 10^5) cells so that the cell density reaches to the optimal 70 ~ 80% confluency at the time of transfection.

Step II. Preparation of Trans-Hi™-DNA Complex and Transfection

For most cell types, the optimal ratio of Trans-Hi™ (μL):DNA (μg) is around 3:1. We recommend this ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity. To ensure the optimal size of Trans-Hi™/ DNA complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and Trans-Hi™ Reagent.

The following protocol is given for transfection in 24-well plates, refer to Table 1 for transfection in other culture formats. The optimal transfection conditions for a majority of adherent cell lines, as well as a general starting point for optimization are given in the standard protocol described below.

All amounts and volumes are given on a per well basis.

1. Add 0.5 ml of complete medium with serum and antibiotics freshly 30 ~ 60 minutes before transfection.
2. Dilute 0.5 μg of DNA into 25 μl of serum-free DMEM with High Glucose. Gently pipette up and down or vortex briefly to mix.
3. Dilute 1.5 μl of Trans-Hi™ reagent into 25 μl of serum-free DMEM with High Glucose. Gently pipette up and down 3 ~ 4 times to mix.

   Never use Opti-MEM to dilute Trans-Hi™ reagent and DNA, as it will interfere with Trans-Hi™/DNA complex formation.
4. Add the diluted Trans-Hi™ reagent **immediately** to the diluted DNA solution all at once**. (Important: do not mix the solutions in the reverse order!)**
5. Immediately pipette up and down 3 ~ 4 times or vortex briefly to mix.
6. Incubate for 10 ~ 15 minutes at room temperature to allow Trans-Hi™/DNA complexes to form.

   Never keep the Trans-Hi™/DNA complex longer than 20 minutes.
7. Add the 50 μl Trans-Hi™/ DNA mixture drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
8. In 12 ~ 18 hrs post transfection remove Trans-Hi™/DNA complex-containing medium and replace with fresh complete serum/antibiotics containing medium.

   For sensitive cells, to lower cytotoxicity, remove Trans-Hi™/DNA complex and replace with complete medium 5 hours after transfection.
9. Visualize/check transfection efficiency 24 to 48 hours post transfection.

Table 1 Recommended Amounts for Different Culture Formats

Culture Dish	Vol. of Culture Medium (mL)	Plasmid DNA (μg)	Dilution Volume (μL)	Trans-Hi™ (μL)
48 well plate	0.3	0.25	2 x 15	0.75
24 well plate	0.5	0.5	2 x 25	1.5
12 well plate	0.75	0.75	2 x 38	2.25
6 well plate	1.0	1.0	2 x 50	3.0
35 mm dish	1.0	1.0	2 x 50	3.0
60 mm dish	2.8	2.5	2 x 100	7.5
10 cm dish	5.0	5.0	2 x 250	15
T75 flask	8.0	9 -18	2 x 400	27 – 54
250mL flask	18	25 – 50	2 x 800	75 - 150

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