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

Trans-Hi™ *In Vitro* DNA Transfection Reagent

----- A Protocol for Generation of rAAV from 293T 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. Trans-Hi™ reagent was shown to generate rAAV with extremely high titers from 293T cells.

Important Transfection Guidelines:

1. The following general protocol is for Generation of rAAV from 293T cell only. Protocols for DNA transfecting mammalian cells lentivirus or adenovirus production are available for download at www.liposomes.com
2. For high titer of rAAV, 293T cell must be healthy. Grow the 293T cell per supplier's instruction.
3. Perform the transfection at high cell density, higher than 80% confluency is strongly recommended.
4. To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics.
5. Use serum-free DMEM with High Glucose to dilute Trans-Hi™ reagent and DNA. The diluent must be serum-free.

Procedures for Transfecting 293T Cells: Cell Seeding (see Table 1):

Cells should be plated 18 to 24 hours prior to transfection so that the optimal ~80% confluency is reached at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection.

Note: 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.

Preparation of Trans-Hi™- DNA Complex and Transfection Procedures

This protocol is given for transfection in 150 mm dish. For other culture formats, scale up or down per culture dish's surface. The optimal transfection conditions are given in the standard protocol described below.

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

1. Make sure the cell confluency is ~80 % at the day of transfection.
2. For each 15 cm dish, add 15 ml of fresh complete medium with serum and antibiotics 30~60 minutes before transfection.
3. Dilute 10 µg of rAAV cis plasmid, 10 µg capsid DNA and 16 µg helper DNA (total 36 µg DNA) in 750 µl serum-free DMEM with high glucose. Vortex gently to mix.
4. Dilute 100 µl of Trans-Hi™ reagent into 750 µl of serum-free DMEM with high glucose. Vortex gently to mix.
   Note: Never use Opti-MEM to dilute DNA and Trans-Hi™ reagent because it will interfere with transfection complex.
5. 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!)
6. Immediately pipette up and down 3 ~ 4 times or vortex briefly to mix followed by ~10 minutes incubation at room temperature.
   Note: Never keep the DNA/Trans-Hi™ complex longer than 20 minutes
7. Add the 1500 µl Trans-Hi™/DNA complex drop-wise onto the medium and homogenize the mixture by gently swirling the plate.
8. Remove DNA/Trans-Hi™ complex-containing medium and replace with fresh complete serum/antibiotics containing medium 5 hours post transfection.
9. Check transfection efficiency and virus titer 24 to 48 hours post transfection. 48 hours gives better titers.

Table 1, Guideline for Seeding 293T Cells Prior to Transfection in Different Culture Formats.

Culture Dishes/Plates	Surface Area (cm2)	Number of Cells to Seed
6-well plate	9.0	$4.0 – 8.0 \times 10^5$
12-well plate	3.5	$1.5 – 3.0 \times 10^5$
24-well plate	2.0	$0.8 – 1.6 \times 10^5$
48-well plate	1.0	$4.0 – 8.0 \times 10^4$
T75 flask	75	$3.0 – 6.0 \times 10^6$
150nm dish	152	$5.0 – 10.0 \times 10^6$
100nm dish	55	$2.2 – 4.4 \times 10^6$
60nm dish	21	$0.9 – 1.8 \times 10^6$
35nm dish	9.5	$3.5 – 7.0 \times 10^5$

This product is for laboratory research ONLY and not for human or diagnostic use

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