Catalog #

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

Trans-Hi™ *In Vitro* DNA Transfection Reagent

-----Protocol for Transfecting 293 and CHO Suspension Cells

Introduction:

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

Important Notes for Transfection:

- Trans-Hi™ transfection reagent is formulated for DNA transfection ONLY! The following standard protocol is for transfecting suspension 293 or CHO cells.
- Protocols for adherent cells, lentivirus, rAAV or adenovirus production are available for download at www.liposomes.com

Advantages:

- Trans-Hi™ Reagent is proven to give very high transfection efficiency in both suspension and adherent cells.
- Add Trans-Hi™ Reagent/DNA complexes directly to cells in standard culture medium and no medium change is required.

Important Guidelines for Transfection:

- For optimal transfection efficiency, dilute Trans-Hi™ Reagent and plasmid DNA ONLY in serum-free DMEM prior to the formation of transfection nanoparticles.
- Make sure your plasmid DNA is of high quality and clean and sterile without contamination of phenol and salt.

Recommended Conditions for Transfection:

Follow the procedure below to transfect suspension 293 or CHO cells in a 30 ml volume. This procedure is totally scalable proportionally to large scales.

Cell volume	30 mL	Prior to transfection
Number of cells to transfect	3E+7 total cells	in standard culture medium
Cell density	1E+6 cells/mL	Must be at 90% viability
Plasmid DNA	~25 µg.	Diluted in 1mL serum free DMEM
Trans-Hi™ Reagent	~60 µL.	Diluted in 1mL serum free DMEM
Volume of Trans-Hi™/DNA	2mL	Prepared in serum free DMEM
Trans-Hi™/DNA ratio	2.4:1 (µL/µg)	Fix this ratio at 2.4:1

Procedures for Transfecting Suspension 293 or CHO Cells:

1. Grow suspension 293 or CHO cells so that at the day of transfection the cell density reaches 3E+7 cells in total 30 mL standard culture medium.
2. At the day of transfection, count cell viability and adjust cell density to 1.0E+6 per mL in total 30 mL (total 3E+7 cells) standard culture medium. Place the shaker flask containing the cells in a 37°C incubator on an orbital shaker.

Important: For best results, make sure to have a single-cell suspension. It may be necessary to vortex the cells vigorously for 10–30 seconds to break down cell clumps. The viability of cells must be >90%.

3. Prepare lipid-DNA complexes for each transfection as follows:
   i) Add 25 µg of plasmid DNA 1mL serum free DMEM. Vortex to mix.
   ii) Add 60 µL of Trans-Hi™ Reagent to 1mL in serum free DMEM. Vortex to mix.
   Note: Never use Opti-MEM to dilute plasmid and Trans-Hi™ Reagent as it may interfere the formation of lipid-DNA complex.
   iii) Add diluted Trans-Hi™ reagent to the diluted DNA right away at all once to obtain total volume of 2 mL transfection mix. Vortex to mix.
   iv) Incubate for 10 minutes at room temperature to allow the formation of DNA-Trans-Hi™ complexes.
4. Add the above-made 2 mL of DNA-Trans-Hi™ complex to each shaker flask containing 30-mL suspension 293 or CHO cells.
   Important: Never leave the DNA-Trans-Hi complex longer than 20 minutes at RT before adding to the cells.
5. Incubate the cells in a 37°C incubator with a humidified atmosphere of 8% CO2 in air on an orbital shaker rotating at 125 rpm.
6. Harvest cells or media (if recombinant protein is secreted) at around 48 hours post-transfection and assay for recombinant protein expression.

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

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