HiLiTran Transfection Reagent Application Guide

Package Content and Storage Conditions

SKU	Components	Storage Condition	Shipping Condition
TT000010	1 vial of HiLiTran Transfection Reagent 0.75 ml	+4℃	Room temperature
TT000010L	1 vial of HiLiTran Transfection Reagent 1.5 ml	+4℃	Room temperature

NOTE: FOR RESEARCH PURPOSES ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USAGE.

Related Products

Expression Plasmid Human,Mouse,Rat
shRNA
siRNA
CRISPR

Introduction

HiLiTran Transfection Reagent is a proprietary formulation for transfecting nucleic acids into a wide range of eukaryotic cells. DNA-HiLiTran complexes must be made in serum-free medium such as Opti-MEM® Reduced Serum Medium and can be added directly to cells in culture medium, in the presence or absence of serum/antibiotic. It is not necessary to remove complexes or change/add medium after transfection. The amount of HiLiTran Reagent required for successful transfection varies depending on the cell type and passage number. Start any new transfection by testing the recommended four concentrations of HiLiTran Reagent to determine an optimum amount.

Required materials

Plasmid DNA
Opti-MEM I Reduced Serum Medium
Eppendorf tubes

Experimental Procedures

A、 HiLiTran DNA Transfection Reagent Protocol

Component	96-well	24-well	6-well
Final DNA per well	100 ng	500 ng	2500 ng
Final HiLiTran Reagent per well	0.2–0.5 µL	1.0–2.5 µL	5.0–12.5 µL

A sample protocol is listed here for experiments performed in 24-well plates. If performing experiments in other cell culture plates, simply multiply the suggested quantities by the relative surface area of your plate.

Step I. Cell Seeding:

① Adherent cells: plated 0.5-2×10^5 cells with 500 µl antibiotic-free medium. Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 70-90%confluency at the time of transfection.

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② Suspension cell: Before preparing and adding the DNA-HiLiTran complex, inculate 4-8×10^5 cells with 500 µl of antibiotic-free medium.

Step Ⅱ. Transfection

For most cell types, the optimal ratio of HiLiTran (uL) : DNA (ug) is around 3:1~2:1 (uL:ug). In order to achieve the highest transfection efficiency and reduce the effect of cytotoxicity, the ratio of DNA to HiLiTran and cell density can be optimized, generally in the range of 1:0.5~1:5 to optimize DNA (µg) and HiLiTran (µl).

The following protocol is for transfection in 24-well plates, refer to Table 1 for transfection in other culture formats.

① **Diluted DNA**: For each well, dilute 0.8 µg of DNA into 50 µl of Opti-MEM I Reduced Serum Medium. Gently pipette up and down or vortex briefly to mix.

② **Diluted HiLiTran**: Add 2.0 µl of HiLiTran reagent into 50 µl of Opti-MEM I Reduced Serum Medium. Gently pipette up and down or vortex briefly to mix. keep at room temperature for 5 minutes.

③ **Add diluted DNA to diluted HiLiTran reagent (1:1 ratio)**. Gently pipette up and down or vortex briefly to mix. Incubate for 20 minutes at room temperature to allow transfection complexes to form. DNA-HiLiTran complexes are stable for 6 hours at room temperature.

④ **Incubate:** Add the mixture prepared in step ③ drop-wise onto cells. Gently rock the plate back and-forth and from side-to-side to distribute the complex evenly. Incubate cells at 37℃.

⑤ **Analyze transfected cell:** Check transfection efficiency 24 to 72 hours post transfection

Table 1. Recommended Amounts for Different Culture Plates

Culture Dish	Area/well	Culture Media		DNA		siRNA	
		Culture Media (plating)	Culture Media (Dilution)	DNA	HiLiTran	siRNA	HiLiTran
96-well	0.3 cm2	100 uL	2 × 25 µl	0.2 µg	0.5 µl	5 pmol	0.25 µl
24-well	2 cm2	500 uL	2 × 50 µl	0.8 µg	2.0 µl	20 pmol	1.0 µl
12-well	4 cm2	1 mL	2 × 100 µl	1.6 µg	4.0 µl	40 pmol	2.0 µl
6-well	10 cm2	2 mL	2 × 250 µl	4.0 µg	10 µl	100 pmol	5 µl
60-mm	20 cm2	5 mL	2 × 0.5 ml	8.0 µg	20 µl	200 pmol	10 µl
10-cm	60 cm2	15 mL	2 × 1.5 ml	24 µg	60 µl	600 pmol	30 µl

B、Co-Transfection of Plasmid DNA and siRNA

Transfect plasmid DNA and siRNA at the same time using HiLiTran Reagent by adding 30 pmol (~0.6 µg) of siRNA per 1 µg of DNA.

C、mRNA Transfection

mRNA can be transfected in a 24-well plate using HiLiTran Reagent by adding 0.5–1 µg of mRNA per wel