HilyMax
Transfection Reagent

Neuro2a cell
Optimum Transfection Condition

Introduction

This protocol shows optimum transfection condition using HilyMax in Neuro2a cells. To tranfect Neuro2a cells in 24-well plate, follow "Optimum Condition for Transfection" and "Transfection Procedure". When using the other vessel, refer to Table 2 and adjust the amounts of cells, medium, DNA and HilyMax in proportion to the relative surface area.

※Important Note※
Optimum Transfection condition is possibly chaged by passage number and culture condition. If transfection efficiency is low by

Optimum Condition for Transfection (for 24-well plate)

Table 1 Optimum condition for tranfection to Neuro2a cells

Cell Density		70%
DNA-HilyMax complex formation	Serum-free medium	30 µl
	DNA	1 µg
	HilyMax	2.0-4.0 µl
	Incubation time	15 min
Medium change after transfection		Necessary

Transfection Procedure (for 24-well plate)

Cell preparation
↓ Adjust the concentration of cells to be 70% confluent in 0.5 ml of growth medium prior to transfection.
Inoculate the cell suspension onto the 24-well plate.
Incubate cells in CO_2 incubator for 24 hr.

Transfection
↓ Form the DNA-HilyMax complex
-Add the serum-free medium(without antibiotics) 30 µl/well in a sterile plastic tube
-Add plasmid DNA 1.0 µg/well and mix by gentle pipetting
-Add HilyMax 2.0-4.0 µl/well and mix by gentle pipetting
-Incubate the mixture of DNA and HilyMax solution at room temperature for 15 minutes
Add DNA-HilyMax complex to cells in each well and mix by gentle shaking the plate
Incubate cells in CO_2 incubator for 18-48 hr
(!) Change the growth medium 4 hours after transfection.

Assay
↓ Measure protein expression

Transfection in Various Vessels

Table 2 Transfection condition in various vessels

Culture of Cells			Formation of DNA-HilyMax complex		
Culture Vessel	Surface Area	Plating Medium	Serum-free Medium	DNA	HilyMax
96 -well	0.3 cm^2	0.1 ml	10 µl	0.2 µg	0.4-0.8 µl
24 -well	1.9 cm^2	0.5 ml	30 µl	1.0 µg	2.0-4.0 µl
12 -well	3.8 cm^2	1.0 ml	60 µl	2.0 µg	4.0-8.0 µl
6 -well	9.2 cm^2	2.0 ml	120 µl	4.0 µg	8.0-16.0 µl
35 -mm	8.0 cm^2	2.0 ml	120 µl	4.0 µg	8.0-16.0 µl
60 -mm	21.0 cm^2	5.0 ml	300 µl	10.0 µg	20.0-40.0 µl
100 -mm	58.0 cm^2	15.0 ml	900 µl	30.0 µg	60.0-120.0 µl

Transfected result by HilyMax

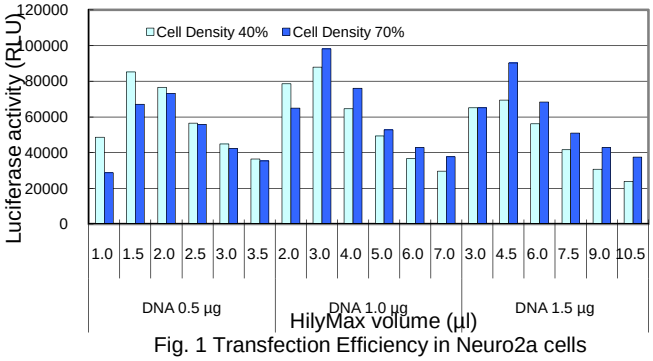

Fig. 1 Transfection Efficiency in Neuro2a cells

Neuro2a cells were incubated for 24 hr and transfected pGL3 control vector (Promega) using HilyMax in each conditions. Transfection efficiency (Luciferase activity) was mesured in 24 hr after transfection.
Neuro2a cells were cultured in MEM medium(Gibco) containing 10%FBS(Gibco) and Non-Essential Amino Acids (Gibco) for about 2 weeks after thawing.

40% confluent：0.75×10^5 cells/well　70% confluent：1.2×10^5 cells/well

Troubleshooting

-Low Transfection Efficiency-
- Change the DNA(µg):HilyMax(µl) ratio to 1:5-1:7.
- Increase the mass of DNA up to 1.5-2.0 times and change the DNA(µg):HilyMax(µl) ratio to 1:2-1:4.

-High cellular Toxicity-
- Decrease the mass of DNA down to half and change the DNA (µg):HilyMax(µl) ratio to 1:2-1:7.

-Check the Material and Condition-
- Was HilyMax Reagent dissolved completely when HilyMax was Prepared?
- Was incubation time of cells after tranfection optimum for cells and plasmid?
- Was DNA-HilyMax complex formed in medium without serum and antibiotics?