HilyMax
Transfection Reagent

Technical Manual

General Information

HilyMax is a newly developed gene transfection reagent. This transfection reagent forms a liposome to be used for highly efficient gene transfection to a wide variety of cells. HilyMax can be applied to siRNA as well. Since serum in the growth medium does not interfere with the transfection using HilyMax, no exchange of the media during the transfection is required. HilyMax does not contain biological components that might interfere with the transfection.

Kit Contents

- HilyMax Reagent	1 tube
- Lipoform Buffer	1.0 ml x 1

Storage Condition

Kit : Store at 0-5°C.

Caution : Store the reconstituted solution of HilyMax at -20°C. It is stable at -20°C for 6 months.

⚠ *For frequent use of the reconstituted HilyMax solution, store at 0-5°C and use the solution within1 month. After thawing the freezed reconstituted HilyMax solution, mix it well by vortex or pipetting.*

Preparation of HilyMax

Add Lipoform Buffer 1.0 ml to HilyMax Reagent, and mix the solution well by vortex for 30 sec.

⚠ *Check HilyMax reagent completely dissolves. If the insoluble gel form remains in the solution, continue the vortex or pipetting until it is completely dissolved.*

Optimum Protocol

To obtain the highest transfection efficiency, it's necessary to confirm the optimum amount of DNA and HilyMax. Optimized transfection protocol and data for 18 cell lines are available. Please access to the following URL.

URL : http://www.dojindo.co.jp/whatsnewsj/newpro/hilymax/hilymax.html

General Protocol

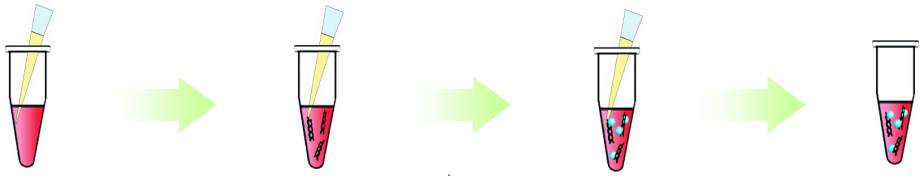
Transfection procedure for a 24-well plate[a)]

1. Cell preparation

Adherent cells
One day before transfection, adjust the concentration of cells to be 40-90% confluent in 0.5 ml of growth medium[b)] for next day and inoculate the cell suspension onto the 24-well plate.

Non-adherent cells
One day before transfection, adjust the concentration of cells to 0.1-1.6 x 10^6 cells in 0.5 ml of growth medium[b)] and inoculate the cell suspension onto the 24-well plate.

2. DNA-HilyMax complex formation[c)]



Add the 30 µl serum-free medium[d)] to a plastic tube.

Add plasmid DNA 0.5-1.5 µg[e)] to the tube and mix by gentle pipetting.

Add HilyMax to the tube and mix by gentle pipetting. Suitable ratio of DNA(µg) to HilyMax (µl) is 1:2-1:7.

Incubate the tube at room temperature for 15 minutes[f)].

3. Addition of complex to cells

Add DNA-HilyMax complex to cell culture well prepared in step 1.

4. Incubation

Incubate the plate at 37°C in CO_2 incubator[g)].

5. Downstream experiment

Measure the reporter gene activity after 18 to 48 hours.

a) Table 2 shows the appropriate amount of DNA, HilyMax and serum-free medium for each size of vessel. Please refer to this table for your use.

b) Serum in growth medium does not interfere the transfection.

c) Optimum amounts of DNA and HilyMax are different in each cell line. Optimize the transfection condition with referring the section of "Optimization of Transfection Condition".

d) Serum and antibiotics in medium interfere with the DNA-HilyMax complex formation. Opti-MEM, DMEM, and MEM can be used for transfection. There is no transfection data available for other media.

e) Use purified plasmid DNA (A_{280}/A_{260}=1.7-1.9). The recommended concentration of DNA for transfection is 0.15-1.00 mg/ml.

f) Over 30 minutes incubation may cause a low transfection.

g) A medium change after 4 hours of incubation increases transfection efficiency and decreases cytotoxicity for most cell lines.

Optimization of Transfection Condition

Optimization of transfection for a 24-well plate

In order to optimize the transfection conditions, examine the amount of DNA, mixing ratio of DNA and HilyMax, cell density and whether medium change is necessary(Figure).

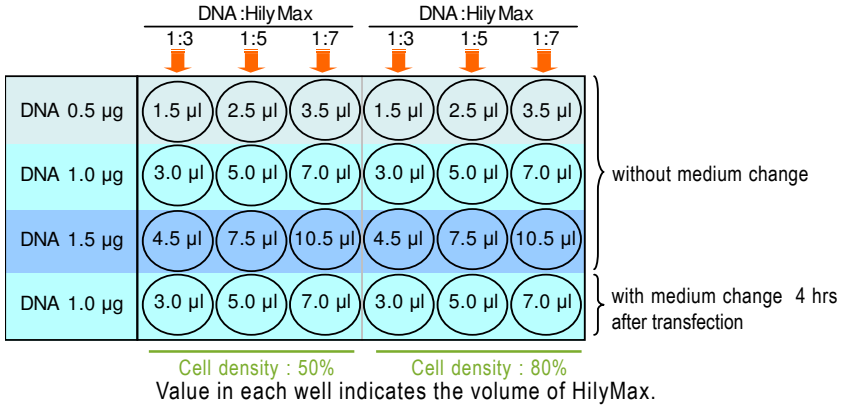


Figure. Example of plate arrangement for optimization

In case using other size of vessels, please multiply the coefficient in Table 1 by the amount of DNA and HilyMax.

Table 1 Coefficient for other vessels

96-well plate : x0.2	12-well plate : x2	6-well plate (35-mm dish) : x4
60-mm dish : x8	100-mm dish : x24	

Transfection Condition for Various Vessels

Table 2 shows suitable amount of medium, DNA, and mixing ratio of DNA and HilyMax solution against each vessel.

Table 2 Transfection condition for various vessels

Culture Vessel	Surface Area	Plating Medium	Serum-Free Medium	DNA	DNA(μg) : HilyMax(μl)
96 -well	0.3 cm^2	0.1 ml	10 μl	0.1-0.3 μg	1:2-1:7
24 -well	1.9 cm^2	0.5 ml	30 μl	0.5-1.5 μg	1:2-1:7
12 -well	3.8 cm^2	1.0 ml	60 μl	1.0-3.0 μg	1:2-1:7
6 -well	9.2 cm^2	2.0 ml	120 μl	2.0-6.0 μg	1:2-1:7
35 -mm	8.0 cm^2	2.0 ml	120 μl	2.0-6.0 μg	1:2-1:7
60 -mm	21.0 cm^2	5.0 ml	300 μl	5.0-15.0 μg	1:2-1:7
100 -mm	58.0 cm^2	15.0 ml	900 μl	15.0-45.0 μg	1:2-1:7

Troubleshooting

Transfection efficiency is low.

1. Is the volume of HilyMax you used enough?
   If no, increase the volume of HilyMax.
2. Is the density of cell you prepared suitable for transfection?
   If no, adjust the density of cell 40-90% confluent.
3. Is HilyMax reagent completly dissolved?
   Check there is no insoluble material.
4. Is the incubation time for the preparation of DNA-HilyMax complex longer than 30 min?
   If yes, shorten the incubation time less than 30min.
5. Does culture medium for DNA-HilyMax complex formation contain serum and/or antibiotics?
   Please use serum free and antibiotics free medium for the complex formation.

Strong cytotoxicity is observed.

1. Reduce the amount of HilyMax and DNA
2. Is the density of cell you prepared suitable for transfection?
   If no, adjust the density of cell 40-90% confluent.

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