M5 Hiper Lipo2000 Transfection Reagent
使用说明书

产品名称	单位	货号
M5 Hiper Lipo2000 Transfection Reagent	0.75 ml	MF135-01
M5 Hiper Lipo2000 Transfection Reagent	1.5 ml	MF135-02

【储存条件】
长期保存，请置于 **4˚C** (切勿结冰冻住)。

【产品简介】
M5 Hiper Lipo2000 Transfection Reagent is a proprietary formulation for transfecting nucleic acids into a wide range of eukaryotic cells. DNA-Lipo 2000 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 Lipo 2000 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 Lipo2000 Reagent to determine an optimum amount.

【产品组分】

	MF135-01	MF135-02
M5 Hiper Lipo2000 Transfection Reagent	0.75 ml	1.5 ml

【实验前准备】

1. Plate cells so they will be 70–90% confluent at the time of transfection.
2. Prepare plasmid DNA-lipid complexes.
3. Add DNA-lipid complexes to cells.

【操作步骤】

A、Lipo2000 DNA Transfection Reagent Protocol（具体方法见后面）

Component	96-well	24-well	6-well
Final DNA per well	100 ng	500 ng	2500 ng
Final Lipo2000 Reagent per well	0.2–0.5 µL	1.0–2.5 µL	5.0–12.5 µL

B、Co-Transfection of Plasmid DNA and siRNA

Transfect plasmid DNA and siRNA at the same time using Lipo2000 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 Lipo2000 Reagent by adding 0.5–1 µg of mRNA per well.

D、Lipo 2000 DNA Transfection Reagent Protocol（具体方法）

Transfect cells according to the following chart. Volumes are given on a per-well basis. Each reaction mix is sufficient for triplicate (96-well), duplicate (24-well), and single well (6-well) transfections, and accounts for pipetting variations. Adjust the amounts of components according to your tissue culture format. For additional information on scaling your transfection reaction, see page 1.

Timeline		Steps
Day 0	1	Seed cells to be 70–90% confluent at transfection
Day 1	2	Dilute four amounts of Lipo2000 Reagent in Opti-MEM® Medium
	3	Dilute DNA in Opti-MEM® Medium
	4	Add diluted DNA to diluted Lipo 2000 Reagent (1:1 ratio)
	5	Incubate
	6	Add DNA-lipid complex to cells
Day 2–4	7	Visualize/analyze transfected cells

Procedure Details

Component	96-well	24-well	6-well
Adherent cells	1–4 × 10^4	0.5–2 × 10^5	0.25–1 × 10^6
Opti-MEM® Medium	25 µL × 4	50 µL × 4	150 µL × 4
Lipo2000 Reagent	1, 1.5, 2, 2.5 µL	2, 3, 4, 5 µL	6, 9, 12, 15 µL
Opti-MEM® Medium	125 µL	250 µL	700 µL
DNA (0.5–5 µg/µL)	2.5 µg	5 µg	14 µg
Diluted DNA Total	25 µL	50 µL	150 µL
Diluted Lipo 2000 Reagent	25 µL	50 µL	150 µL
Incubate for 5 minutes at room temperature.			

Component	96-well	24-well	6-well
DNA-lipid complex per well	10 µL	50 µL	250 µL
Final DNA used per well	100 ng	500 ng	2500 ng
Final Lipo 2000 Reagent used per well	0.2–0.5 µL	1.0–2.5 µL	5.0–12.5 µL
Incubate cells for 1–3 days at 37°C. Then analyze transfected cells.			

【举例：质粒 DNA 的转染】

对大多数细胞来说，DNA(μg)与 Lipo2000 (μl)的比例为 1:2~1:3。转染时高的细胞密度可以得到高的转染效率和表达水平，并能减少细胞毒性。

1. 以 24 孔板为例

贴壁细胞： 转染前一天，用 500 μl 不含抗生素的培养基接种 0.5～2×10^5 细胞，使之第二天能达到 70-90%汇合。

悬浮细胞：在准备 DNA-Lipo2000 复合物之前，用 500 μl 不含抗生素的培养基接种 4～8×10^5 细胞即可。

2. 对每个转染样品，进行以下操作

a. 在eppendorf管里分别加入50 μl Opti-MEM I ReLipced Serum Medium和0.8 μg DNA轻柔混匀，制成DNA稀释液。

b. 在另一个eppendorf管里分别加入50μl Opti-MEMI ReLipced Serum Medium和2.0 μl Lipo2000 (注意用前先混匀)，轻柔混匀，制成Lipo2000 稀释液，室温静置5分钟。

c. 将DNA稀释液和Lipo2000稀释液混合，轻柔混匀，室温静置20分钟， 形成DNA-Lipo2000复合物。DNA-Lipo2000复合物在室温下可稳定存在6小时。

3. 将DNA-Lipo2000复合物加入到接种好的细胞中，将培养板轻轻地前后摇动，使复合物分散均匀。

4. 在37℃ CO_2培养箱中培养4-6小时后更换培养基,继续培养18～48小时。

5. 如要筛选稳定细胞株，则在转染24小时后将细胞按照1:10或更高的比例接种到新鲜培养基中，第二天加入选择性培养基进行筛选。

质粒DNA转染的优化 为达到最高的转染效率和降低细胞毒性的影响，可以对DNA和Lipo2000的比例以及细胞密度进行优化，一般在1:0.5~1:5的范围内优化DNA (μg)和Lipo2000 (μl) 的比例。

不同细胞培养板中转染时培养基、核酸及 ***Lipo2000*** 用量

细胞培养板	每孔面积	培养基用量		DNA转染		siRNA	
		铺板培养基用量	稀释培养基用量				
96-well	0.3 cm^2	100 uL	2 × 25 μl	0.2 μg	0.5 μl	5 pmol	0.25 μl
24-well	2 cm^2	500 uL	2 × 50 μl	0.8 μg	2.0 μl	20 pmol	1.0 μl
12-well	4 cm^2	1 mL	2 × 100 μl	1.6 μg	4.0 μl	40 pmol	2.0 μl
6-well	10 cm^2	2 mL	2 × 250 μl	4.0 μg	10 μl	100 pmol	5 μl
60-mm	20 cm^2	5 mL	2 × 0.5 ml	8.0 μg	20 μl	200 pmol	10 μl
10-cm	60 cm^2	15 mL	2 × 1.5 ml	24 μg	60 μl	600 pmol	30 μl

Lip2000 转染试剂用于不同细胞转染时用量参考（以 96 孔板为例）

细胞型号	培养基	每孔细胞数	DNA的量	转染试剂量
293H	DMEM	3x104	0.2μg	0.5μL
293FT	DMEM	3x104	0.2μg	0.5μL
293E	DMEM	3x104	0.2μg	0.5μL
293F	DMEM	3x104	0.2μg	0.5μL
COS7	DMEM	1.5x104	0.4μg	0.5μL
hela	DMEM	2x104	0.3μg	0.5μL
Caco2	MEM	3.5x104	0.3μg	0.75μL
BHK21	MEM	2x104	0.2μg	0.5μL
CHO-DG44	DMEM+HT+pro	2x104	0.5μg	0.5μL
RAW264.7	DMEM	3x104	0.2μg	0.5μL
MCF7	MEM/NEAA+0.01mg/mL insulin + sodium pyruvat	2x104	0.1μg	0.25μL
SW480	IMDM	3x104	0.4μg	0.5μL
MDCK	DMEM	4x104	0.6μg	1μL
CHO-K1	IMDM+Pro	3x104	0.2μg	0.5μL
HepG2	DMEM	3x104	0.5μg	0.75μL
A549	DMEM	2x104	0.3μg	0.5μL
NIH/3T3	DMEM	1.5x104	0.1μg	0.75μL
vero	DMEM	3x104	0.3μg	0.75μL
sf9	SIM SF	5x104	0.4μg	0.75μL

常见细胞的转染效率（仅供参考，实验条件不同转染效率会有差别）：

细胞种类	HEK293	HCT 116	WRL -68	HepG2	NIH/3T3	THP-1	Hela	MCF-7	293T	TS cell	HO1980	A549
转染效率	>80%	>80%	~80%	~80%	~80%	>50%	>80%	>80%	>80%	>60%	>60%	>80%
细胞种类	MEF	Chok1	Hep3 B	C2C12	Neuro-2a	HUVEC	MDCK	Hep2C	WEHI	B50	Calu1	L929
转染效率	>50%	>50%	>80%	>80%	>70%	>80%	>80%	>80%	>80%	>70%	>70%	>70%

【备注】
本产品仅供科研使用。在确认产品质量出现问题时，本公司承诺为客户免费更换等量的质量合格产品。