Lipo2000® Transfection Reagent

保存： 2–4℃保存一年。（避免冷冻）

产品说明

Lipo2000®是一种新型的阳离子脂质体转染试剂。适合于将核酸(DNA 和 RNA)转染入真核细胞，具有低细胞毒性；对多种类型的细胞和培养板都具有高转染效率；转染时血清的存在不影响转染效率的优点。

适用范围：贴壁细胞和悬浮细胞（哺乳动物细胞系）的转染。

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℃ CO2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. 如果要筛选稳定细胞株，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

Lip 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	3×104	0.2μg	0.5μL
293FT	DMEM	3×104	0.2μg	0.5μL
293E	DMEM	3×104	0.2μg	0.5μL
293F	DMEM	3×104	0.2μg	0.5μL
COS7	DMEM	1.5×104	0.4μg	0.5μL
hela	DMEM	2×104	0.3μg	0.5μL
Caco2	MEM	3.5×104	0.3μg	0.75μL
BHK21	MEM	2×104	0.2μg	0.5μL
CHO-DG44	DMEM+HT+pro	2×104	0.5μg	0.5μL
RAW264.7	DMEM	3×104	0.2μg	0.5μL
MCF7	MEM/NEAA+0.01mg/mL insulin + sodium pyruvat	2×104	0.1μg	0.25μL
SW480	IMDM	3×104	0.4μg	0.5μL
MDCK	DMEM	4×104	0.6μg	1μL
CHO-K1	IMDM+Pro	3×104	0.2μg	0.5μL
HepG2	DMEM	3×104	0.5μg	0.75μL
A549	DMEM	2×104	0.3μg	0.5μL
NIH/3T3	DMEM	1.5×104	0.1μg	0.75μL
vero	DMEM	3×104	0.3μg	0.75μL
sf9	SIM SF	5×104	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	Hep3B	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%

Lipo2000® Transfection Reagent

Description

Lipo2000® is a newly developed and proprietary reagent for the transfection of nucleic acids into eukaryotic cells. Lipo2000® has the following advantages:

The highest transfection efficiency in many cell types and formats.
DNA-Lipo2000® complexes can be directly added to cells in culture medium (with or without serum).
It is not necessary to remove DNA-Lipo2000™ complexes or change medium following transfection.
The complexes can be removed after 4-6 hours by replacing with refresh medium (optional)

Contents and Storage

Lipo2000® is supplied in liquid form at a concentration of 1mg/ml. Store at 4°C. **DO NOT FREEZE**.

Product Qualification

Lipo2000® has been extensively tested by transfection of HEK293 cells with an EGFP reporter containing plasmid. Lipo2000® is free of microbial contamination.

Important Guidelines

Follow these guidelines when performing transfections:

1. The ratio of DNA (in µg) : Lipo2000® (in µl) to use when preparing complexes should be 1:2 to 1:3 for most cell lines. To transfect 0.5 -2 X10^5 cells in a 24-well format, use 0.8-1 µg DNA and 2-3 µl of Lipo2000™. Optimizing transfection by varying DNA/Lipo2000® ratio is possible.
2. It is **CRITICAL** to transfect cells at high cell density. 90-95% confluence the time of transfection is recommended to obtain high efficiency and expression levels and to minimize decreased cell growth associated with high transfection activity. Lower cell densities are suitable with optimization of conditions. Take care to maintain a standard seeding protocol between experiments because transfection efficiency is dependent on culture confluence.
3. **DO NOT** add antibiotics to media during transfection as this will cause cell death.

For better results, you may choose to:

Use Opti-MEM I medium to dilute Lipo2000® prior to complexing with DNA. Other media without serum (e.g.DMEM) may be used to dilute Lipo2000®，but transfection efficiency may be compromised.

Note: Some serum-free formulations can inhibit Lipo2000™ mediated transfection, for example: CD 293， 293 SFM II， and VP-SFM etc.

Transfection Procedure for 24-Well Format

For adherent cells: One day before transfection, plate cells in growth medium (without antibiotics) so that they will be 90-95% confluent at the time of transfection (0.5-2 x 10^5 cells/well for a 24-well plate).

For suspension cells: On the day of transfection just prior to preparing complexes, plate 4-8 x 10^5 cells/500 µl of growth medium (without antibiotics) in a 24-well plate.

1. For each transfection sample， prepare DNA-Lipo2000™ complexes as follows:
   - Dilute DNA in 50 µl of Opti-MEMI Reduced Serum Medium without serum (or other medium without serum). Mix gently.
   - Mix Lipo2000™ gently before use， then dilute the appropriate amount in 50 µl of Opti-MEM I Medium (or other medium without serum). Mix gently and incubate for 5 minutes at room temperature. Note: Combine the diluted Lipo2000® with the diluted DNA within 30 minutes. Longer incubation times may decrease activity. If DMEM is used as a diluent for the Lipo2000™， mix with the diluted DNA **within 5 minutes**.
   - After the 5 minute incubation, combine the diluted DNA with the diluted Lipo2000®

(total volume is 100 µl). Mix gently and incubate for 20 minutes at room temperature to allow the DNA-Lipo2000® complexes to form. The solution may appear cloudy, but this will not inhibit the transfection. Note: DNA-Lipo2000® complexes are stable for at least 5 hours at room temperature.

3. Add the 100 µl of DNA-Lipo2000® complexes to each well. Mix gently by rocking the plate back and forth.
4. Incubate the cells at 37°C in a CO_2 incubator for 24-48 hours until they are ready to assay for transgene expression. It is not necessary to remove the complexes or change the medium; however， growth medium may be replaced after 4-6 hours without loss of transfection activity.

For stable cell lines: Passage the cells at a 1:10 or higher dilution into fresh growth medium 24 hours after transfection. Add selective medium the following day.

For suspension cells: Add PMA and/or PHA (if desired) 4 hours after adding the DNA-Lipo2000® complexes to the cells. Tip: For Jurkat cells， adding PHA-L and PMA at final concentrations of 1 µg/ml and 50 ng/ml，respectively， enhances CMV promoter activity and gene expression. For K562 cells， adding PMA alone is sufficient to enhance promoter activity.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Lipo2000®， DNA， cells， and medium used in proportion to the difference in surface area (see table below). With automated， high-throughput systems, larger complexing volumes are recommended for transfections in 96-well plates. Note: You may perform rapid 96-well plate transfections (plate cells and transfect simultaneously) by adding a suspension of cells directly to complexes prepared in the plate. Prepare complexes and add cells at twice the cell density as in the basic protocol in a 100 µl volume. Cells will adhere as usual in the presence of DNA-Lipo2000™ complexes.

Culture Vessel	Surface Area per Well (cm^2)	Relative Surface Area (vs. 24-well)	Volume of Plating Medium	DNA (µg) and Dilution Volume (µl)	Lipo2000™ (µl) and Dilution Volume (µl)
96-well	0.3	0.2	100 µl	0.2 µg in 25 µl	0.5 µl in 25 µl
24-well	2	1	500 µl	0.8 µg in 50 µl	2.0 µl in 50 µl
12-well	4	2	1 ml	1.6 µg in 100 µl	4.0 µl in 100 µl
35-mm	10	5	2 ml	4.0 µg in 250 µl	10 µl in 250 µl
6-well	10	5	2 ml	4.0 µg in 250 µl	10 µl in 250 µl
60-mm	20	10	5 ml	8.0 µg in 0.5 ml	20 µl in 0.5 ml
10-cm	60	30	15 ml	24 µg in 1.5 ml	60 µl in 1.5 ml

Note: Surface areas are determined from actual measurements of tissue culture vessels.

Optimizing Transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying DNA and Lipo2000® concentrations， and cell number. Make sure that cells are greater than 90% confluent and vary DNA (µg) : Lipo2000® (µl) ratios from 1:0.5 to 1:5.