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Lipo2000® Transfection Reagent

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(每次转染一定要进行预实验，来摸索最佳条件,尤其是转染试剂的最佳用量!)

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 0.5～2×105 细胞，使之第二天细胞融合度达到 90%最合适。（融合度 100%过高，培养基很快发黄，影响转染效率）

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。

*在转染前 1 小时，换 37℃预热过不含双抗的完培 800ul/孔（通常含 FBS 5% ，此处血清不影响转染效率。但未预热的培养基会影响转染效率）。

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 Reclipped 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 Reclipped Serum Medium 和 1.5～2.0 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 小时后更换以上预热的培养基，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转染	
		铺板培养基用量	稀释培养基用量	DNA	转染试剂用量	RNA	转染试剂用量
96-well	0.3 cm^2	100 uL	2 × 25 μl	0.1 μg	0.2～0.5 μl	5 pmol	0.2～0.25 μl
24-well	2 cm^2	500 uL	2 × 50 μl	0.5 μg	0.6～2.5 μl	20 pmol	0.8～1.0 μl
12-well	4 cm^2	1 mL	2 × 100 μl	1 μg	2～4.5 μl	40 pmol	1.5～2.0 μl
6-well	10 cm^2	2 mL	2 × 250 μl	2～4 μg	5～10 μl	100 pmol	4～5 μl
60-mm	20 cm^2	5 mL	2 × 0.5 ml	4～8 μg	10～20 μl	200 pmol	7～10 μl
10-cm	60 cm^2	15 mL	2 × 1.5 ml	12～24 μg	30～60 μl	600 pmol	25～30 μl

保存： 2-4℃ 保存一年。（避免冷冻）

细胞转染注意事项：

细胞 /DNA/siRNA 每次都不同，建议每次转染一定要进行预实验来摸索最佳条件，尤其是转染试剂的最佳用量！

1) 转染试验失败原因：DNA/siRNA，转染试剂和细胞。转染效率低要先排除一下是否是 siRNA 的问题，如果 siRNA 无效，换再好的转染试剂也没意义，可以用带荧光标记的 control siRNA 做一下看看，转进去的话会有荧光的。确实转染效率低，可以考虑换转染试剂；毒性大则要减少转染试剂的用量。
2) 细胞的种类和状态影响较大：转染时细胞必须处于良好生长状态，转染时细胞的密度一般铺板率在达到 70—80% 最好（此时细胞处于对数生长期）；
3) 如果是贴壁细胞，应保证贴壁在 12 ～ 24 小时在进行转染，否则细胞转染容易脱壁。对于贴壁生长细胞，一般要求在转染前一日，必须应用胰酶处理成单细胞悬液，重新接种于培养皿或瓶，转染当日的细胞密度以 70-90% （贴壁细胞）或 2×10^6-4×10^6 细胞 /ml （悬浮细胞）为宜，最好在转染前 4h 换一次新鲜培养液。
4) 质粒的大小，质量和用量对转染效率很关键。
5) 转染时注意脂质体和用量，过量的话对细胞毒性大也容易失败。
6) 转染作用 6 小时一定记得要换含血清的培养基
7) 培养基以及洗涤细胞和稀释用的培养基要无血清、无双抗。
8) 转染试剂对个别细胞可能有一定毒性，在转染过程由于提高细胞的通透性因而不能在培养答基中添加抗生素。
9) 高纯度的 DNA 或 RNA 可获得较高的转染效率！用于转染的质粒 DNA 必须无蛋白质，无 RNA 和其他化学物质的污染， OD260/280 比值应在 1.8 以上。血清中含有大量的蛋白质，在转染过程中，带负电的蛋白质可能干扰阳离子脂质体对核酸的吸附，影响转染效率。另外，使用脂质体等转染试剂时，由于含血清转染会将血清中的蛋白带入细胞，引发细胞毒性，导致转染效率降低，故用无血清培养基转染效果更好！
10) 培养基中的血清：在开始准备 DNA 和转染试剂稀释液时要使用无血清的培养基，因为血清会影响复合物的形成。其实，只要在 DNA- 转染试剂复合物形成时不含血清，在转染过程中是可以使用血清的。
11) 培养基中的抗生素：抗生素是影响转染的培养基添加物。这些抗生素一般对于真核细胞无毒，但阳离子脂质体试剂增加了细胞的通透性，使抗生素可以进入细胞。这降低了细胞的活性，导致转染效率降低。
12) 一般在转染 24-48h，靶基因即在细胞内表达。根据不同的实验目的， 24-48h 后即可进行靶基因表达的检测实验。
13) 如若建立稳定的细胞系，则可对靶细胞进行筛选，根据不同基因载体中所含有的抗性标志选用相应的药物，常用的真核表达基因载体的标志物有潮霉素和新霉素等。
14) 建议设置阳性对照和阴性对照。

常见细胞的转染效率（荧光显微镜检测数据仅供参考，实验条件不同转染效率会有差别）

细胞种类	HEK293	293T	293F	HCT 116	WRL -68	HepG2	NIH/3T3	THP-1	Hela	MCF-7	CV-1
转染效率	99%	99%	99%	80%	80%	~80%	~80%	>50%	94%	>80%	70%
细胞种类	CHO-S	CHO	COS-7L	Chok1	Hep3B	C2C12	Neuro-2a	HUVEC	MDCK	Hep2C	WEHI
转染效率	97%	96%	99%	50%	80%	>80%	>70%	>80%	60%	>80%	>80%
细胞种类	HT-1080	SKBR3	Vero	MEF	BE(2)C	A549	L929	HO1980	Calu1	TS cell	B50
转染效率	81%	50%	85%	50%	77%	>80%	>70%	>60%	>70%	>60%	>70%

转染效率的测定方法

1)实时定量 PCR 检测; 2)Western Blot 检测; 3)流式细胞术
4)荧光显微镜观察：转染含有荧光蛋白基因质粒 DNA，通过荧光显微镜观察荧光的强弱以及荧光的效率。

Lipo 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

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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** aclivity. Lower cell densities are suitable with oplimizalion of condilions. Take care to maintain a standard seeding orotocol between experiments because lransfeclion eficlency is dependent on culture confuence.

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). 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 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 diluted DNA within **5 minutes**.
- After the 5 minutes 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.

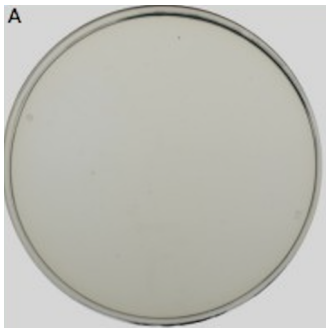
Optimizing Transfection

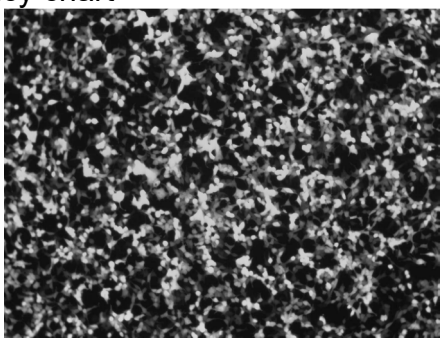
To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying DNA and Lipo2000® concentrations and cell number. Make sure that cells are greater than 90% confluent and vary DNA (µg): (Lipo2000®µl) ratios from 1:0.5 to 1:5.

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Catalog #	#F055	CAS#	
Product Name	Lipo2000 trasfection reagent		
Solubility	Soluble in water		

Microscope and 293T transfection efficiency chart





Items	Lot #DCM90916	Specifications (≥1 mg) #F029
Appearance	colorless solution	colorless solution
Absorption Maximum	N/A	N/A
Extinction Coefficient($cm^{-1}M^{-1}$)	N/A	N/A
HPLC Purity	99.78%	≥ 98% @254 nm detection
Storage Condition		<4 ºC, desiccated, avoid light
Shipping Condition		Room temperature

QA：杜冰冰

QC：谢辉



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