

## Lipo6000™转染试剂

产品编号	产品名称	包装
C0526-0.5ml	Lipo6000™转染试剂	0.5ml
C0526-1.5ml	Lipo6000™转染试剂	1.5ml
C0526-7.5ml	Lipo6000™转染试剂	5×1.5ml

### 产品简介:

- Lipo6000™转染试剂(Lipo6000™ Transfection Reagent)是一种非常高效的新型转染试剂,达到了国际最主流转染试剂的转染效果。适用于把质粒、siRNA或其它形式的核酸包括DNA、RNA、寡核苷酸、以及核酸蛋白复合物或带负电荷的蛋白转染到真核细胞中,也可以用于活体动物的核酸转染以用于基因治疗。
- Lipo6000™转染试剂对于常见的哺乳动物细胞具有非常高的转染效率、重复性好、操作简单、无明显的细胞毒性,并且对于贴壁细胞和悬浮细胞都适用。贴壁细胞转染试剂的比较和选择请参考: <http://www.beyotime.com/support/lipo.htm>。
- Lipo6000™转染试剂的使用方法和常用的Lipofectamine® 2000 Reagent基本一致。并且经过对HEK293T、Hela、NIH3T3、HEK293FT、CHO等细胞的测试,转染效率也和Lipofectamine® 2000 Reagent相当甚至略高。
- Lipo6000™转染试剂不仅适用于质粒、siRNA等单一成分的细胞转染,也适合多个质粒或者质粒与siRNA等的组合转染。
- Lipo6000™转染试剂转染过表达质粒后,通常24-48小时后达到较高的蛋白表达水平,并且很多情况下蛋白表达量在转染后48小时显著高于转染后24小时;转染siRNA通常3-5天后对于目的基因的下调水平会比较理想。
- Lipo6000™转染试剂转染细胞时,基本不受细胞培养液中的血清和抗生素的影响,即可以在血清和抗生素存在的情况下进行细胞转染。但为了取得最佳的转染效果,推荐转染时使用不含抗生素的含血清的细胞培养液。
- Lipo6000™转染试剂的转染效果可以通过转染表达EGFP等荧光蛋白的质粒进行快速鉴定。
- Lipo6000™转染试剂与Lipofectamine® 2000 Reagent转染效果比较请参考图1-6。

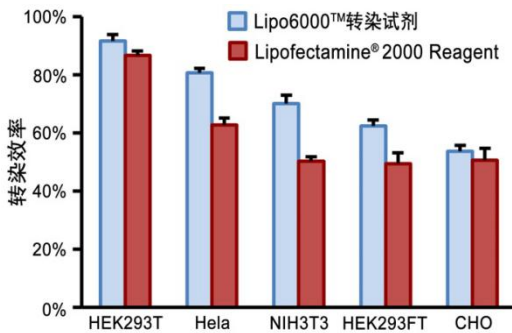


图1. Lipo6000™转染试剂与Lipofectamine® 2000 Reagent转染效率的比较。仅转染试剂不同,其余条件一致。

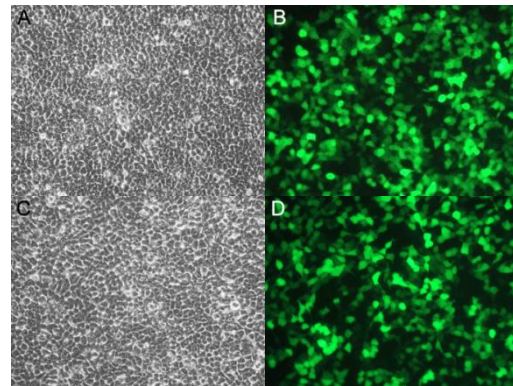


图2. Lipo6000™转染试剂(A, B)与Lipofectamine® 2000 Reagent (C, D)用EGFP表达质粒转染HEK293T细胞后的实拍效果图。

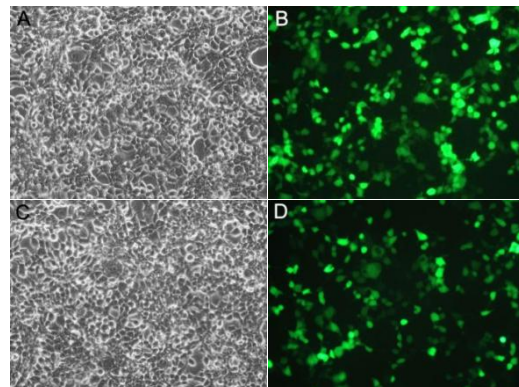
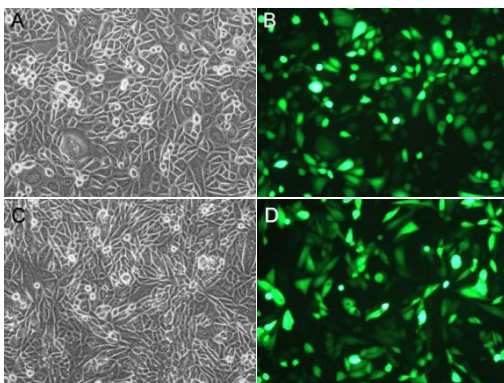


图3. Lipo6000™ 转染试剂(A, B)与 Lipofectamine® 2000 Reagent (C, D)用EGFP表达质粒转染Hela细胞后的实拍效果图。

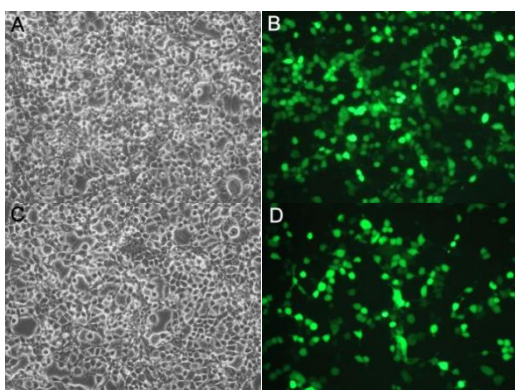


图5. Lipo6000™ 转染试剂(A, B)与 Lipofectamine® 2000 Reagent (C, D)用EGFP表达质粒转染HEK293FT细胞后的实拍效果图。

- 对于六孔板，一个包装的C0526-0.5ml、C0526-1.5ml和C0526-7.5ml转染试剂大约可以分别转染100个、300个和1500个孔；对于24孔板，一个包装的C0526-0.5ml、C0526-1.5ml和C0526-7.5ml转染试剂大约可以分别转染500个、1500个和7500个孔。

**包装清单：**

产品编号	产品名称	包装
C0526-0.5ml	Lipo6000™转染试剂	0.5ml
C0526-1.5ml	Lipo6000™转染试剂	1.5ml
C0526-7.5ml	Lipo6000™转染试剂	5×1.5ml
—	说明书	1份

**保存条件：**

4°C保存。长期不使用可以-20°C保存。

**注意事项：**

- 使用高纯度的DNA或RNA有助于获得较高的转染效率。对于质粒，可以使用碧云天生产的质粒大量抽提试剂盒(D0026)进行抽提，以保证可以获得较高的转染效率。
- 转染前细胞必须处于良好的生长状态。
- 需自备不含抗生素的无血清培养液或Opti-MEM®培养液或普通的DMEM培养液。
- Lipo6000™转染试剂不能vortex或离心，宜缓慢晃动混匀。
- Lipo6000™转染试剂使用后请立即盖好盖子，避免长时间暴露在空气中，影响转染效率。
- 本产品仅限于专业人员的科学研究用，不得用于临床诊断或治疗，不得用于食品或药品，不得存放于普通住宅内。
- 为了您的安全和健康，请穿实验服并戴一次性手套操作。

**使用说明：**

**1. DNA转染：**

- 细胞培养(以六孔板为例，其它培养板或培养皿可参考六孔板)：在转染前一天(18-24小时)按照每孔约20-70万细胞(具体的细胞数量据细胞类型、大小和细胞生长速度等而定)接种到六孔板内进行培养，使第二天细胞密度能达到约70-90%。
- 在进行下述转染步骤前，把培养有细胞的六孔板每孔换成2ml新鲜培养液(含有血清，不含抗生素)。可以使用含有血清并含有抗生素的新鲜培养液，但抗生素的存在对于有些细胞容易导致转染后出现一定的细胞毒性。
- 参考下表，对于待转染的六孔板中每一个孔的细胞，取两个洁净无菌离心管，分别加入125μl不含抗生素和血清的DMEM培养液(高糖DMEM或低糖DMEM均可)或Opti-MEM® Medium，然后于其中一管加入2.5μg质粒DNA，并用枪轻轻吹打混匀；另一管加入5μl Lipo6000™转染试剂，用枪轻轻吹打混匀，请特别注意不可Vortex或离心。室温静置5分钟后(通常最长不宜超过25分钟)，将含有DNA的培养液用枪轻轻加入含Lipo6000™转染试剂的培养液中，轻轻颠倒离心管或者用枪轻轻吹打混匀，室温静置5分钟(室温存放6小时内稳定)。

	96-well	48-well	24-well	12-well	6-well	6cm dish	10cm dish
Lipo6000™转染试剂	0.2μl	0.5μl	1μl	2μl	5μl	10μl	30μl
无血清培养液或Opti-MEM® Medium	5μl	12.5μl	25μl	50μl	125μl	250μl	750μl

图4. Lipo6000™ 转染试剂(A, B)与 Lipofectamine® 2000 Reagent (C, D)用EGFP表达质粒转染NIH3T3细胞后的实拍效果图。

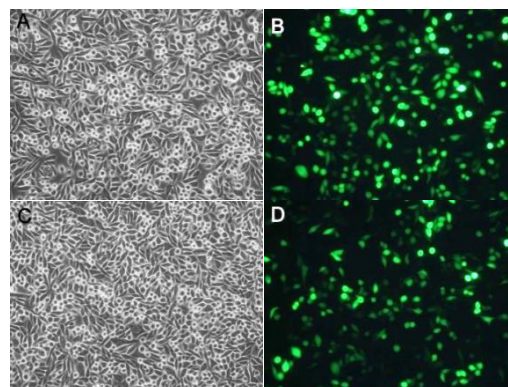


图6. Lipo6000™ 转染试剂(A, B)与 Lipofectamine® 2000 Reagent (C, D)用EGFP表达质粒转染CHO细胞后的实拍效果图。



DNA	100ng	250ng	500ng	1µg	2.5µg	5µg	15µg
无血清培养液或Opti-MEM® Medium	5µl	12.5µl	25µl	50µl	125µl	250µl	750µl
稀释好的Lipo6000™转染试剂和DNA分别室温静置放置5分钟，随后两者混合并混匀再室温静置放置5分钟							
每孔加入的混合物的量	10µl	25µl	50µl	100µl	250µl	500µl	1500µl
按照上述用量每孔均匀滴加Lipo6000™转染试剂和DNA的混合物，4-6小时后更换培养液或直接继续培养							

注1: 对于六孔板中一个孔的细胞, Lipo6000™转染试剂的用量可以在3-12.5µl范围内进行适当调节, DNA用量建议固定在2.5µg, 但也可以在1-4µg的范围内进行适当调节。通常质粒用量(µg)和Lipo6000™(µl)的用量比例为1:2或1:3比较常用, 如有必要可以在1:0.5-1:5的范围内优化转染效果, 上表推荐的比例为1:2, 此时Lipo6000™的用量相对较少, 既经济又高效。最佳的转染条件, 因不同的细胞类型和培养条件而有所不同, 可以在上述推荐范围内自行优化转染条件。

注2: 质粒的浓度宜控制在0.5-5µg/µl范围内。

注3: 对于多个孔转染相同数量相同质粒的情况可以把每个孔所需的Lipo6000™转染试剂和DNA混合物分别配制, 然后一起混合在同一个离心管内, 后续混匀并孵育5分钟后, 可以按照推荐用量滴加到细胞培养器皿内。

注4: 对于其它培养板或培养器皿, 各种试剂的用量可以按照细胞培养器皿的培养面积按比例进行换算。如果转染寡核苷酸或RNA等可以参考转染DNA的条件进行。

- d. 无论是贴壁细胞还是悬浮细胞, 按照六孔板每孔250µl Lipo6000™转染试剂-DNA混合物的用量, 均匀滴加到整个孔内, 随后轻轻混匀。
- e. 为达到最高的转染效率, 细胞在转染后培养4-6小时后宜更换为新鲜的完全培养液(对于Hela细胞, 推荐在转染4小时后更换培养液, 对于NIH3T3、CHO、HEK293T和HEK293FT细胞, 推荐在转染6小时后更换培养液)。
- f. 继续培养约24-48小时后, 即可用适当方式检测转染效果, 例如荧光检测、Western、ELISA、报告基因等, 或加入适当的筛选药物如G418等进行稳定细胞株的筛选。

## 2. siRNA转染:

- a. 细胞培养(以六孔板为例, 其它培养板或培养皿可参考六孔板): 在转染前一天(18-24小时)按照每孔约20-70万细胞(具体的细胞数量据细胞类型、大小和细胞生长速度等而定)接种到六孔板内进行培养, 使第二天细胞密度能达到约30-50%。
- b. 在进行下述转染步骤前, 把培养有细胞的六孔板每孔换成2ml新鲜培养液(含有血清, 不含抗生素)。可以使用含有血清并含有抗生素的新鲜培养液, 但抗生素的存在对于有些细胞容易导致转染后出现一定的细胞毒性。
- c. 参考下表, 对于待转染的六孔板中每一个孔的细胞, 取两个洁净无菌离心管, 分别加入125µl不含抗生素和血清的DMEM培养液(高糖DMEM或低糖DMEM均可)或Opti-MEM® Medium, 然后于其中一管加入100pmol siRNA, 并用枪轻轻吹打混匀; 而另一管加入5µl Lipo6000™转染试剂, 用枪轻轻吹打混匀, 请特别注意不可Vortex或离心。室温静置5分钟后(通常最长不宜超过25分钟), 将含有siRNA的培养液用枪轻轻加入含Lipo6000™转染试剂的培养液中, 轻轻颠倒离心管或者用枪轻轻吹打混匀, 室温静置5分钟(室温存放6小时内稳定)。

	96-well	48-well	24-well	12-well	6-well	6cm dish	10cm dish
Lipo6000™转染试剂	0.2µl	0.5µl	1µl	2µl	5µl	10µl	30µl
无血清培养液或Opti-MEM® Medium	5µl	12.5µl	25µl	50µl	125µl	250µl	750µl
siRNA	4pmol	10pmol	20pmol	40pmol	100pmol	200pmol	600pmol
无血清培养液或Opti-MEM® Medium	5µl	12.5µl	25µl	50µl	125µl	250µl	750µl
稀释好的Lipo6000™转染试剂和siRNA分别室温静置放置5分钟, 随后两者混合并混匀再室温静置放置5分钟							
每孔加入的混合物的量	10µl	25µl	50µl	100µl	250µl	500µl	1500µl
按照上述用量每孔均匀滴加Lipo6000™转染试剂和siRNA的混合物, 4-6小时后更换培养液或直接继续培养							

注1: 对于六孔板中一个孔的细胞, Lipo6000™转染试剂的用量可以在2.5-7.5µl范围内进行适当调节, siRNA用量可以在50-250pmol的范围内进行适当调节。通常siRNA用量(pmol)和Lipo6000™(µl)的用量比例为20:1, 如有必要可以在10:1-40:1的范围内优化转染效果, 上表推荐的比例为20:1, 此时Lipo6000™的用量相对较少, 既经济又高效。最佳的转染条件, 因不同的细胞类型和培养条件而有所不同, 可以在上述推荐范围内自行优化转染条件。

注2: siRNA的推荐浓度为20µM, 常用的浓度范围为10-50µM。

注3: 对于多个孔转染相同数量相同质粒的情况可以把每个孔所需的Lipo6000™转染试剂和siRNA混合物分别配制, 然后一起混合在同一个离心管内, 后续混匀并孵育5分钟后, 可以按照推荐用量滴加到细胞培养器皿内。

注4: 对于其它培养板或培养器皿, 各种试剂的用量可以按照细胞培养器皿的培养面积按比例进行换算。如果转染寡核苷酸或RNA等可以参考转染DNA的条件进行。

- d. 无论是贴壁细胞还是悬浮细胞, 按照六孔板每孔250µl Lipo6000™转染试剂-siRNA混合物的用量, 均匀滴加到整个孔内, 随后轻轻混匀。
- e. 为达到最高的转染效率, 细胞在转染后培养4-6小时后宜更换为新鲜的完全培养液(对于Hela细胞, 推荐在转染4小时后更换培养液, 对于NIH3T3、CHO、HEK293T和HEK293FT细胞, 推荐在转染6小时后更换培养液)。

f. 继续培养3-5天后, 即可用适当方式检测siRNA对于靶基因的下调效果, 例如qPCR、Western、ELISA、报告基因等。

### 常见问题:

#### 1. 转染效率低:

- 优化质粒与Lipo6000™转染试剂比例, 对于难转染的细胞, 可适当加大质粒用量。
- 应用高纯度、无菌、无污染物的质粒进行转染, DNA纯度方面A<sub>260</sub>/A<sub>280</sub>比值要接近1.8, 通常宜控制在1.8-1.9范围内, 偏低则有可能有蛋白污染, 偏高则有可能有RNA污染。可以使用碧云天生产的质粒大量抽提试剂盒(D0026)进行抽提, 以保证可以获得较高的转染效率。
- 贴壁细胞转染时状态良好, 细胞密度达30-50%时才可进行转染, 过稀可能影响转染效率, 细胞密度达到50-90%时通常不会影响转染效率。不同细胞的最佳转染密度需要自行摸索。悬浮细胞 宜在对数生长期进行转染。
- 需使用无抗生素和无血清培养液配制Lipo6000™转染试剂和质粒或siRNA等的混合物。
- 转染后培养时间不足, 而被误以为转染效率偏低。不同细胞转染后至显著表达所需要培养的时间通常为24-48小时。
- 检查细胞是否有支原体感染, 支原体感染会影响细胞增殖, 并很可能影响转染效率。
- 如果没有检测到目的蛋白表达, 应该仔细核对转染质粒的测序结果, 确保测序结果和读码框完全正确。
- 如果靶基因的敲减(knockdown)效果欠佳, 应该考虑尝试设计不同的siRNA。

#### 2. 细胞毒性较大:

- 缩短转染时间, 在转染后较短时间内更换新鲜的细胞培养液。
- 减少质粒用量, 按照比例减少 Lipo6000™转染试剂。
- 检查是否转染时细胞密度太低。

### 附录:

常用多孔板和培养皿的尺寸、培养面积、细胞培养量和推荐的培养体积等相关数据表:

Multiple Well Plates or Dishes	Single Well Only for Plates					
	Diameter (Bottom, mm)*	Growth Area (cm <sup>2</sup> )*	Average Cell Yield	Total Well Volume (ml)	Working Volume (ml)	Recommended Volume (ml)
6 well	34.8	9.5	9.5 × 10 <sup>5</sup>	16.8	1.9-2.9	2
12 well	22.1	3.8	3.8 × 10 <sup>5</sup>	6.9	0.76-1.14	1
24 well	15.6	1.9	1.9 × 10 <sup>5</sup>	3.4	0.38-0.57	0.5
48 well	11.0	0.95	9.5 × 10 <sup>4</sup>	1.6	0.19-0.285	0.25
96 well	6.4	0.32	3.2 × 10 <sup>4</sup>	0.36	0.10-0.20	0.1
384 well	2.7	0.056	5.6 × 10 <sup>3</sup>	0.112	0.025-0.050	0.030
1536 well	1.63 × 1.63**	0.025	2.5 × 10 <sup>3</sup>	0.0125	0.005-0.010	0.010
3.5 cm dish	34	9	9.0 × 10 <sup>5</sup>	NA	1.8-2.7	2
6 cm dish	52	21	2.1 × 10 <sup>6</sup>	NA	4.2-6.3	5
10 cm dish	8.4	55	5.5 × 10 <sup>6</sup>	NA	11-16.5	12
15cm dish	14	152	1.5 × 10 <sup>7</sup>	NA	30.4-45.6	35
24.5cm dish	22.4 × 22.4**	500	5.0 × 10 <sup>7</sup>	NA	100-150	120

\*Diameter and growth area may vary depending on the manufacturer, and the listed sizes are from Corning.

\*\*These wells or dishes are square.

### 相关产品:

产品编号	产品名称	包装
C0508	磷酸钙法细胞转染试剂盒	>200次
C0511	DEAE-Dextran细胞转染试剂盒	>200次
C0518-1ml	Lipo293F™转染试剂	1ml
C0518-10ml	Lipo293F™转染试剂	10ml
C0518-100ml	Lipo293F™转染试剂	100ml
C0521-0.5ml	Lipo293™转染试剂	0.5ml
C0521-1.5ml	Lipo293™转染试剂	1.5ml
C0521-7.5ml	Lipo293™转染试剂	5×1.5ml
C0526-0.5ml	Lipo6000™转染试剂	0.5ml
C0526-1.5ml	Lipo6000™转染试剂	1.5ml
C0526-7.5ml	Lipo6000™转染试剂	5×1.5ml
C0533-0.5ml	Lipo8000™转染试剂	0.5ml

C0533-1.5ml	Lipo8000™转染试剂	1.5ml
C0533-7.5ml	Lipo8000™转染试剂	5×1.5ml
C0551-0.5ml	LipoInsect™转染试剂	0.5ml
C0551-1.5ml	LipoInsect™转染试剂	1.5ml
C0551-7.5ml	LipoInsect™转染试剂	5×1.5ml

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