EnoGeneFec™ 2200 Transfection Reagent

Catalog Number: EGF2200-500, EGF2200-1000, EGF2200-1500

Amount:500μl□ 1000μl□ 1500μl□

Storage/Stability: Store at 4℃/1 year

Introduction

EnoGeneFec™ 2200 Transfection Reagent comprises of unique formulations of polycations and liposomes representing a new class of transfection reagent designed for primary mammalian cells to get outstanding transfection results, which will guarantee higher transfection efficiency and lower cytotoxicity for almost all primary cell lines. EnoGeneFec™ 2200 Transfection Reagent offers significant advantages over many other transfection methods.

EnoGeneFec™ 2200 Transfection Reagent offers:

- High transfection efficiency of plasmids, antisense oligonucleotides, siRNA in almost all primary cell types
- Fast procedure — transfection-complex formation in just 20 minutes
- Decreased cytotoxicity

Kit Contents

Cat:	EnoGeneFec™ 2200 Transfection Reagent
EGF2200-500	500μL
EGF2200-1000	1.0mL
EGF2200-1500	2.0mL

Important Notes

Required amount of EnoGeneFec™ 2200 Transfection Reagent

For initial optimization experiments, transfect a monolayer of cells that is 70–90% confluent, using 2:1~ 5:1 ratios of EnoGeneFec™ 2200 Transfection Reagent (μl) to DNA (μg), respectively. For most cell types, the EnoGeneFec™ 2200 Transfection Reagent: DNA (2:1) ratio provides excellent transfection level. For some hard-to-transfect cells, the EnoGeneFec™ 2200 Transfection Reagent: DNA (5:1) ratio is recommended.

Cell culture

A healthy cell culture lays the foundation for successful transfection. Different cells or cell lines have very specific media, serum, and supplement requirements. Low passage number (<50 splitting cycles) ensures that the cell genotype has not become altered. We recommend subculturing cells 24 hours before transfection. This provides normal cell metabolism and increases the likelihood of DNA uptake.

Effect of serum

We do not recommend using serum during complex formation between EnoGeneFec™ 2200 Transfection Reagent and plasmid DNA, as serum may inhibit complex formation.

Plasmid DNA quality

It is critical to accurately determine the plasmid DNA concentration using 260nm absorption. DNA content must be determined by 260nm absorption (estimates of DNA content based on the intensity of gel bands are not sufficiently accurate). Determine the DNA purity using a 260 nm/280 nm ratio; the ratio should be 1.8.

Procedures

Use the following conditions as guidelines to transfect primary mammalian cells in a 6-well or 35mm dish format. For other culture vessels, please refer to Table 1.

Table 1: Reagent Quantities for Different Culture Vessels

Culture Vessel	Surface area per well (cm^2)	Typical total volume of medium (ml)	Starting mass of DNA (μg) in medium volume (μl)	EnoGeneFec™ 2200 in medium volume	Transfection medium vol.
96 well	0.3	0.1-0.2	0.2 μg in 20 μl	0.4-1.0 μl in 20 μl	50 μl
24 well	2	0.5	(0.2-)1.0 μg in 25 μl	2-5 μl in 25 μl	0.4 ml
12 well	4	1.0	(0.5-)2.0 μg in 100 μl	4-10 μl in 100 μl	0.6 ml
6 well	10	2.0	(1.0-)5.0 μg in 100 μl	10-25 μl in 100 μl	0.8 ml
35 mm	10	2.0	(1.0-)5.0 μg in 100 μl	10-25 μl in 100 μl	0.8 ml
60 mm	20	5.0	(3.0-)10.0 μg in 500 μl	20-50 μl in 500 μl	2.4 ml
10 cm	60	10.0	(8.0-)20.0 μg in 1500 μl	40-100 μl in 800μl	6.4 ml

For *in vitro* primary adherent cells

1. 18 to 24 hours prior to transfection, trypsinize and adjust the cell concentration, seed cells at a density of 1-3 x 10^5 cells per well in 2.0ml of appropriate growth medium (with serum and antibiotics if cells are cultured in the presence of them). Incubate the cells at 37˚C in a CO_2 incubator until cells are 70% to 90% confluent at the time of transfection.

2. For each transfection sample, prepare the complexes as follows:

Solution A: Dilute 2.0 μg of DNA into 100 μl of serum-free, antibiotic-free medium.

Solution B: Vortex EnoGeneFec™ 2200 Transfection Reagent thoroughly prior use, then dilute 10 μl of EnoGeneFec™ 2200 Transfection Reagent in 100 μl serum-free, antibiotic-free medium.

Incubate Solution A and B at room temperature for 5 minutes.

3. Combine the solutions, mix gently to ensure uniform distribution and incubate for 20 minutes at room temperature. NOTE: *Complexes are stable at room temperature for 3-5 hours.*

4. Add 0.8 ml of serum-free, antibiotic-free medium to EnoGeneFec™-DNA complex. Mix solution gently.

5. Remove growth medium from the cells and add 1.0 ml of EnoGeneFec™-DNA solution to the each well containing cells.

6. After 5-8 hours, remove transfection solution and add 2.0 ml of the appropriate growth medium (with serum and antibiotics). Incubate the cells at 37˚C in a CO_2 incubator for a total of 24-72 hours.

7. Assay cell extracts or phenotype for gene activity 24-72 hours after the start of transfection depending on the cell types and promoter activity. To make stable cell lines: Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours post transfection. Selection medium can be added the following day if desired.

For *in vitro* primary suspension cells

1. Just prior to preparing complexes, plate 3-5 x 10^5 suspension cells in 0.8ml of serum free medium without antibiotics. Since transfection efficiency is sensitive to culture confluence, it is important to maintain a standard seeding protocol from experiment to experiment.

2. For each transfection sample, prepare the complexes as follows:

Solution A: Dilute 2.0 μg of DNA into 100 μl of serum-free, antibiotic-free medium.

Solution B: Vortex EnoGeneFec™ 2100Transfection Reagent thoroughly prior use, then dilute 10 μl of EnoGeneFec™ 2100Transfection Reagent in 100 μl serum-free, antibiotic-free medium.

Incubate Solution A and B at room temperature for 5 minutes.

3. Combine the solutions, mix gently to ensure uniform distribution and incubate for 20 minutes at room temperature. NOTE: Complexes are stable at room temperature for 3-5 hours.

4. Add 0.2ml of the EnoGeneFec™-DNA solution into each well containing suspension cells in 0.8ml serum-free, antibiotic-free medium.

5. After 5-8 hours, add 0.1ml of FBS directly into each vessel.. Incubate the cells at 37˚C in a CO_2 incubator for a total of 24-72 hours.

6. Assay cell extracts or phenotype for gene activity 24-72 hours after the start of transfection depending on the cell types and promoter activity. To make stable cell lines: Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours post transfection. Selection medium can be added the following day if desired.

Storage and stability

EnoGeneFec™ 2200 Transfection Reagent is provided in 1μg/μLconcentration. It is shipped at room temperature and is stabilized for extended storage at +4°C for one year when very tightly closed.

EnoGeneFec™ 2200 转染试剂
（EnoGeneFec™ 2200 Transfection Reagent）

一、 产品介绍

EnoGene 新推出的 EnoGeneFec™ 转染试剂以最高的转染效率、使用方便、细胞毒性小、生物可降解为设计宗旨，EnoGene 的新型配方克服了常见的阳离子或脂质体转染试剂带来的细胞毒性作用，更适合做长效和瞬时转染。使用这种新的转染试剂操作方便，可用于转染质粒、线性双链 DNA、反义寡核苷酸及 RNAi 等，在实际使用中获得了非常理想的效果。

EnoGeneFec™ 2200 是针对原代细胞设计的转染试剂，可用于体外转染质粒、线性双链 DNA、反义寡核苷酸及 RNAi 等。对常用的原代细胞转染效率可达 60%以上。

EnoGeneFec™ 2200 可以形成微小的（平均大小约 100-400nm）单层脂质体，靠静电作用结合到DNA的磷酸骨架上以及带负电的细胞膜表面，并与寡核苷酸等能形成稳定的较小的纳米胶体颗粒，通过细胞"内吞作用"进入细胞，能吸收溶酶体的H^+，在复合体中形成酸性环境使核酸酶失活，保护DNA免受核酸酶的降解。进入细胞后，复合体囊泡肿胀破裂，将DNA释放到细胞质中，实现基因转染。。

EnoGeneFec™ 2200 与其他转染试剂相比无论在转染效果和实验操作上都有明显的优势，主要表现为：

- ●转染效率很高，对大多数原代贴壁或原代悬浮细胞使用效果综合评价明显高于其它常用转染试剂
- ●细胞毒性低，需要转染较大剂量的 DNA 时，毒性明显低于其它常用转染试剂
- ●操作简单，以最短的时间完成转染，转染试剂-DNA 复合物的形成时间只需 20min

二、 试剂盒组分

组分	EnoGeneFec™ 2200 Transfection Reagent
EGF2200-500	0.5ml
EGF2200-1000	1ml
EGF2200-1500	1.5ml

三、 操作步骤（以 6 孔板为例）

对于原代贴壁细胞：

1. 转染前 18-24 小时使用完全培养基在 6 孔细胞培养板，每孔接种 2ml 细胞培液（根据细胞培养条件，可含血清及抗生素），约为 1-3×10^5个细胞，转染前细胞密度应达到 70－90%。若使用其他的培养板或培养皿，可以参照表 1 中提供的孔（或皿）表面积和体积相应的调整细胞接种数、EnoGeneFec™ 2200 加量。

表 1：细胞培养孔（或皿）表面积和体积相应的调整细胞接种数、EnoGeneFec™ 2200 加量

培养板	表面积（cm^2/孔or皿）	可容纳培养基体积（ml/孔or皿）	DNA（或其他核苷酸类成分）用量（μg）	EnoGeneFec™ 2200 用量	待加的无血清、无抗生素的培养基体积
96 孔	0.3	0.1-0.2	0.2μg 溶于 20μl 培养基	0.4μl 溶于 20μl培养基	50μl
24 孔	2	0.5	0.2-1 μg溶于 25μl 培养基	2-4 μl 溶于 25μl 培养基	0.4ml

12 孔	4	1.0	0.5-2.0 μg 溶于 100 μl 培养基	4-8 μl 溶于 100 μl 培养基	0.6ml
6 孔	10	2.0	1.0-5.0 μg 溶于 100 μl 培养基	10-20 μl 溶于 100 μl 培养基	0.8ml
35 mm	10	2.0	1.0-5.0 μg 溶于 100 μl 培养基	10-20 μl 溶于 100 培养基	0.8ml
60mm	20	5ml	3.0-10.0 μg 溶于 500 μl 培养基	10-20 μl 溶于 500 培养基	2.4ml
10-cm	60	10ml	8.0-20.0 μg 溶于 1.5ml 培养基	90-200 μl 溶于 800 μl 培养基	6.4ml

2. 转染复合物的制备：

溶液 A：将 5μg 质粒 DNA（或其他核苷酸类成分）加入到 100 μl**无血清、无抗生素的培养基，**在 1.5ml 无菌 EP 管中混匀后，室温放置 5 分钟。

溶液 B：EnoGeneFec™ 2200 在使用前请震荡混匀。将 10-20μl EnoGeneFec™ 2200 加入到 100 μl **无血清、无抗生素的培养基中，**在 1.5ml 无菌 EP 管中混匀，室温放置 5 分钟。

将溶液 A 加入到溶液 B 中，轻轻混匀，室温放置 20 分钟，获得约 200 μl 转染复合物 （注：该转染复合物在室温下 5 小时内是稳定的）。

3. 将 800 μl **无血清、无抗生素的培养基加入到**上述的 200 μl 转染复合物中，轻轻混匀，获得约 1.0ml 转染复合物溶液。将培养板中的培养基弃去，将获得的 1.0ml 转染复合物溶液加入到培养孔中覆盖细胞。

4. 于 5-8 小时后，弃去每孔中的转染复合物溶液，加入 2.0ml 完全培养基（根据细胞培养条件，可含血清及抗生素），37℃孵育转染细胞 18-24 小时。

6. 收集细胞用于分析。如要建立稳定转染，于转染 24 小时后将细胞按 1:10 传代至新鲜培养基中（根据细胞培养条件，可含血清及抗生素），传代次日可以换用选择培养基。

对于原代悬浮细胞：

1. 在制备转染复合物之前 1 小时，接种 0.8ml **无血清、无抗生素的培养基细胞悬液**（含 3-5×10^5个细胞）至 6 孔细胞培养板中。若使用其他的培养板或培养皿，可以参照表 1 中提供的孔（或皿）表面积和体积相应的调整细胞接种数、EnoGeneFec™ 2200 加量。

2. 转染复合物的制备：

溶液 A：将 5μg 质粒 DNA（或其他核苷酸类成分）加入到 100 μl**无血清、无抗生素的培养基，**在 1.5ml 无菌 EP 管中混匀后，室温放置 5 分钟。

溶液 B：EnoGeneFec™ 2200 在使用前请震荡混匀。将 10-20μl EnoGeneFec™ 2200 加入到 100 μl **无血清、无抗生素的培养基中，**在 1.5ml 无菌 EP 管中混匀，室温放置 5 分钟。

将溶液 A 加入到溶液 B 中，轻轻混匀，室温放置 20 分钟，获得约 200 μl 转染复合物 （注：该转染复合物在室温下 5 小时内是稳定的）。

3. 将上述的 200 μl转染复合物滴加到第一步接种了 0.8ml **无血清、无抗生素的培养基细胞悬液** （含 3-5×10^5个细胞）的培养孔中，轻轻混匀。

4. 于 5-8 小时后，培养孔中加入 0.1ml 血清（可含抗生素），37℃孵育转染细胞 18-24 小时。

5. 收集细胞用于分析。如要建立稳定转染，于转染 24 小时后将细胞按 1:10 传代至新鲜培养基中（根据细胞培养条件，可含血清及抗生素），传代次日可以换用选择培养基。

四、 注意事项

1. 质粒 DNA 的质量

使用高纯度的质粒 DNA 也是转染试验中的关键因素。为了保证试验的结果，建议对提取的质粒 DNA 的量和纯度进行检测。DNA 含量(μg/mL)=50×(260 nm 的读数)×稀释倍数，另外通过检测质粒 DNA 在 260nm 和 280nm 的 OD 值的比值（OD260/OD280）估计核酸的纯度，OD260/OD280=1.8 说明 DNA 样本纯度较高。

2. 转染细胞的要求

细胞的传代次数是影响转染效果的重要因素，推荐使用在 5 代以内的原代细胞进行转染试验。

3. 血清的影响

在 EnoGeneFec™ 2200 和 DNA 形成转染复合物的过程中不能添加血清。

4. EnoGeneFec™ 2200 的用量

为了达到更高的转染效率，对于接种细胞密度在 70%-90%之间的样本，可通过预试验在 EnoGeneFec™ 2200（μl）：DNA（μg）=1：1 - 5：1 之间选择最佳的比例。EnoGeneFec™ 2200（μl）：DNA（μg）的推荐比例为 2：1 或 5：1。对于一般细胞，2：1 即可获得理想转染效果；对于较难转染的细胞，建议使用 5：1。

五、储存

EnoGeneFec™ 2200 以 1 μg/ μl浓度液体形式提供，保存在 4℃。常温运输。

保存期：一年