EnoGeneFec™ 2300 Transfection Reagent

Catalog Number: EGF2300-500, EGF2300-1000, EGF2300-1500

Amount:500μl□ 1000μl□ 1500μl□

Storage/Stability: Store at 4℃/1 year

Introduction

EnoGeneFec™ 2300 Transfection Reagent comprises of unique formulations of polycations and liposomes representing a new class of transfection reagent designed for outstanding *in vivo* transfection results, which will guarantee higher transfection efficiency and lower cytotoxicity. EnoGeneFec™ 2300 Transfection Reagent offers significant advantages over many other transfection methods.

EnoGeneFec™ 2300 Transfection Reagent offers:

- High transfection efficiency of plasmids, antisense oligonucleotides, siRNA
- Fast procedure — transfection-complex formation in just 20 minutes
- Decreased cytotoxicity and biodegradable
- Suitable for *in vivo* transfection

Kit Contents

Cat:	EnoGeneFec™ 2300 Transfection Reagent
EGF2300-500	500μL
EGF2300-1000	1.0mL
EGF2300-1500	2.0mL

Important Notes

Required amount of EnoGeneFec™ 2300 Transfection Reagent

For most cell types, the EnoGeneFec™ 2300 Transfection Reagent: DNA (2:1) ratio provides excellent transfection level. For some hard-to-transfect cells, the EnoGeneFec™ 2300 Transfection Reagent: DNA (5:1) ratio is recommended.

Effect of serum

We do not recommend using serum during complex formation between EnoGeneFec™ 2300 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.

***In vivo* transfection**

For *in vivo* transfection, disease animal model such as nude mice human cancer xenograft model shall be chose according to customer's research purpose. The *in vivo* dosage of oligos (including plasmids, antisense oligonucleotides, siRNA, etc) may be adjusted to the effective dosage based on customer's previous studies. For most condition, the EnoGeneFec™ 2300 Transfection Reagent (2:1 or 5:1) ratio provides excellent transfection level. The route of administration of EnoGeneFec™ 2300 Transfection Reagent: DNA complex may be designed according to future clinical application route such as *i.v., i.m., i.p,* or intratumor injection, etc.

Procedures

Use the following conditions as guidelines for in vivo transfection.

The EnoGeneFec™ 2300 Transfection Reagent: DNA ratio is recommended according to the types of oligos (including plasmids, antisense oligonucleotides, siRNA, etc) as in Table 1 for customer's optimization.

Table 1: Reagent Quantities for Different Oligos

Types of oligos	EnoGeneFec™ 2300 Transfection Reagent: DNA ratio
Plasmids	2-5：1
Antisense oligonucleotides	2-5：1
siRNA	2-5：1

For nude mice human cancer xenograft model gene therapy by intratumor injection as example:

1) Nude mice human cancer xenograft model preparation

Under SPF conditions, the tumor particles or Log phase cancer cells are inoculated s.c. of nude mice right axilla. The xenografted tumor was measured the length and width by vernier caliper. Nude mice are randomly divided into several groups when the bearing tumor reached 100 mm^3 volume. Tumor length and width were measured twice every week to dynamic evaluate the anticancer activity of the tested compounds.

Tumor volume (TV) was calculated by the following formula:

$TV = 1/2 \times a \times b^2$

a and b represents length and width, respectively.

2) Preparation of EnoGeneFec™ 2300 Transfection Reagent: DNA complex

Solution A: Dilute 10.0 μg of DNA into 50 μl of serum-free, antibiotic-free medium.

Solution B: Vortex EnoGeneFec™ 2300 Transfection Reagent thoroughly prior use, then dilute 50 μl of EnoGeneFec™ 2300 Transfection Reagent in 50 μl serum-free, antibiotic-free medium.

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

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.*

3) Complex injection

The above 100 ul EnoGeneFec™ 2300 Transfection Reagent-DNA complex is injected directly into the tumor after degermation of the local tumor.

4) Multiple dosing

If multiple dosing is required, repeat step 2) and 3). After period of oligos DNA administration, nude mice are sacrificed and tumors are isolated by surgery for analysis.

Storage and stability

EnoGeneFec™ 2300 Transfection Reagent is provided in 1 μg/μL concentration. It is shipped at room temperature and is stabilized for extended storage at +4°C for one year when very tightly closed.

EnoGeneFec™ 2300 转染试剂
（EnoGeneFec™ 2300 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™ 2300 是针对动物体内转染（*in vivo transfection*）设计的转染试剂，可用于动物体内转染质粒、反义寡核苷酸及 RNAi 等。对常用的细胞转染效率可达 60%以上。

EnoGeneFec™ 2300 进入动物体内后可以形成微小的（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™ 2300 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，生物可降解，专为动物体内转染设计
- ●细胞毒性及对动物的毒性均低，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™ 2300 Transfection Reagent
EGF2300-500	0.5ml
EGF2300-1000	1ml
EGF2300-1500	1.5ml

三、 操作步骤

1. 质粒 DNA（或其他核苷酸类成分）用量与 EnoGeneFec™ 2300 的使用比例推荐见表 1：

表 1：体内转染试验 EnoGeneFec™ 2300 用量参照表

核酸类型	EnoGeneFec™ 2300 用量： 质粒 DNA（或其他核苷酸类成分）用量
质粒	2-5：1
反义寡聚核酸	2-5：1
RNAi	2-5：1

以小鼠瘤内注射或瘤周注射进行基因治疗为例：

2. 肿瘤细胞株或瘤组织块接种于小鼠皮下，定期用游标卡尺测量移植瘤直径，待肿瘤生长至一定体积后将动物随机分组给药。肿瘤体积(tumor volume ,TV)的计算公式为：

TV = 1/2×a×b^2 其中a、b分别表示长宽。

3. 转染复合物的制备：

溶液 A：将 10μg 质粒 DNA（或其他核苷酸类成分）加入到 20 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™ 2300 在使用前请震荡混匀。将 20μl EnoGeneFec™ 2300 加入到 10 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 50 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）。

4. 将 50 μl **无血清、无抗生素的培养基加入到**上述的 50 μl转染复合物中，轻轻混匀，获得约 100 μl转染复合物溶液。

5. 瘤体局部消毒后，将步骤 4 获得的 100μl 转染复合物直接注射或多点注射到荷瘤小鼠瘤内或瘤周。

6. 动物继续饲养观察，如需要多次给药，则转染方法按步骤 3、步骤 4 进行。定期用游标卡尺测量移植瘤直径，计算肿瘤体积。

7. 处死动物，手术剥取瘤块进行分析。

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. 血清的影响。

在 EnoGeneFec™ 2300 和 DNA 形成转染复合物的过程中不能添加血清。

3. EnoGeneFec™ 2300 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™ 2300（μl）：DNA（μg）=1：1 - 5：1 之间选择最佳的比例。EnoGeneFec™ 2300（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™ 2300 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