

Transfection Reagent

EnoGeneFec 2000 Transfection Reagent

CATALOG #
AAA269062

PRODUCT SYNONYMS

Transfection Reagent; EnoGeneFec 2000 Transfection Reagent; Transfection Reagent reagent

PRODUCT SPECIFICATIONS

Concentration	1ug/ul (varies by lot)
Important Notes	Required amount of EnoGeneFec™ 2000 Transfection Reagent For initial optimization experiments, transfect a monolayer of cells that is 70–90% confluent, using 2:1~ 5:1 ratios of EnoGeneFec™ 2000 Transfection Reagent (ul) to DNA (ug), respectively. For most cell types, the EnoGeneFec™ 2000 Transfection Reagent: DNA (2:1) ratio provides excellent transfection level. For some hard-to-transfect cells, the EnoGeneFec™ 2000 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™ 2000 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.
Preparation and Storage	It is stabilized for extended storage at 4 degree C for one year when very tightly closed.

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™ 2000 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) 0.0 µg in 25 µl	2-5 µl in 25 µl	0.4 ml
12 well	4	1.0	(0.5-2) 0.0 µg in 100 µl	4-10 µl in 100 µl	0.6 ml
6 well	10	2.0	(1.0-5) 0.0 µg in 100 µl	10-25 µl in 100 µl	0.8 ml
35 mm	10	2.0	(1.0-5) 0.0 µg in 100 µl	10-25 µl in 100 µl	0.8 ml
60 mm	20	5.0	(3.0-10) 0.0 µg in 500 µl	20-50 µl in 500 µl	2.4 ml
10 cm	60	10.0	(8.0-20) 0.0 µg in 1500 µl	40-100 µl in 800 µl	6.4 ml

Application Data

1. 18 to 24 hours prior to transfection, trypsinize and adjust the cell concentration, seed cells at a density of 1-3 x 10⁵ 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 degree C in a CO₂ 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 ug of DNA into 100 ul of serum-free, antibiotic-free medium.Solution B: Vortex EnoGeneFec™ 2000 Transfection Reagent thoroughly prior use, then dilute 10 ul of EnoGeneFec™ 2000 Transfection Reagent in 100 ul 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 degree C in a CO₂ 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.Use the following conditions as guidelines to transfect adherent mammalian cells in a 6-well or 35mm dish format. For other culture vessels, please refer to Table 1.

ADDITIONAL INFORMATION

Related Product Information for Transfection Reagent reagent

Introduction:
EnoGeneFec™ 2000 Transfection Reagent comprises of unique formulations of polycations and liposomes representing a new class of transfection reagent designed for adherent mammalian cells to get outstanding transfection results, which will guarantee higher transfection efficiency and lower cytotoxicity for almost all adherent cell lines. EnoGeneFec™ 2000 Transfection Reagent offers significant advantages over many other transfection methods.

EnoGeneFec™ 2000 Transfection Reagent offers:

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

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.