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GreenFect™ Transfection Reagent

Cat. #: M0004 Size: 0.75 ml/1.5 ml

Introduction:

GreenFect™ Transfection Reagent is a lipid-based transfection reagent that forms a complex with DNA or RNA, and transports the complex into a variety of adherent and suspension cell lines. This reagent delivers superior transfection efficiency and improved cell viability for the widest range of hard-totransfect and common cells. GreenFect™ Transfection Reagent has been tested to work the same efficiency as Lipofectamine® 3000 Reagent, and used for the transfection of both DNA and RNA into eukaryotic cells even in the presence of serum.

Key Features:

1. Superior transfection efficiency for a broad range of cell lines, especially for difficult-to-transfect cells.
2. Does not require removal of serum or culture medium.
3. Does not require washing or changing of medium after transfection.
4. Low cytotoxicity.

Package Information

Components	M0004-01	M0004-02
GreenFect™ Reagent (Component A)	0.75 ml	1.5 ml
Enhancer Reagent (Component B)	0.75 ml	1.5 ml

Protocols

Use the following procedure to transfect DNA into mammalian cells in a 24-well format. For other formats, see Scaling Up or Down Transfections. All amounts and volumes are given on a per well basis. Transfect cells at high cell density for high efficiency, high expression levels, and to minimize cytotoxicity. Optimization may be necessary (see Optimizing Transfection).

1. **Adherent cells**: One day before transfection, plate 0.5-2×10^5 cells in 500 µl of growth medium without antibiotics so that cells will be 70-90% confluent at the time of transfection.

Suspension cells: Just prior to preparing complexes, plate 4-8×10^5 cells in 500 µl of growth medium without antibiotics.

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

a. Mix GreenFect™ Reagent (Component A) gently before use, then dilute 1 µl of GreenFect™ Reagent in 25 µl of Opti-MEM® I Medium. Incubate at room temperature.

b. Dilute 0.5 µg DNA in 25 µl of Opti-MEM® I Reduced Serum Medium without serum (or other medium without serum). Then add 1 µl of Enhancer Reagent (Component B). Mix gently and incubate at room temperature.

c. Add diluted DNA/Enhancer Reagent mixture to the diluted GreenFect™ Reagent (total volume = 50 µl). Mix gently and incubate for 10-15 minutes at room temperature.

3. Add the 50 µl of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.

4. Incubate cells at 37°C in a CO_2 incubator for 2-4 days. Then, analyze transfected cells. Medium may be changed after 4-6 hours.

Optimizing Transfection

To obtain the highest transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density as well as DNA and GreenFect™ Reagent concentrations. Make sure that cells are greater than 90% confluent and vary DNA (µg): GreenFect™ Reagent (µl): Enhancer Reagent (µl) ratios from 1:1:2 to 1:4:2.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of GreenFect™ Reagent, Enhancer Reagent, nucleic acid, cells, and medium used in proportion to the relative surface area, as shown in the table.

Table 1. A Guideline for Optimal DNA Per Well in Different Culture Formats

Culture Dishes	Surface Area (cm^2)	Plating medium volume	Dilution medium volume	DNA	Green-Fect™	Enhancer Reagent
96-well	0.3	100 µl	2× 5 µl	0.1 µg	0.15~0.3 µl	0.2 µl
24-well	2	500 µl	2× 25 µl	0.5 µg	0.75~1.5 µl	1 µl
12-well	4	1 ml	2× 50 µl	1.0 µg	1.5~3.0 µl	2 µl
6-well	10	2 ml	2× 125 µl	2.5 µg	3.75~7.5 µl	5 µl

Storage: GreenFect™ Transfection Reagent is stable for up to 12 months at 4°C.