E-Fect DNA Transfection Reagent

Cat. No.: LE-10203

Package: 250µl (liquid form)

Storage: Store at -20°C (non-frost-free).

Introduction

E-Fect is a cationic polymer gene transfection reagent. It has several unique features necessary for efficient transfection, such as DNA condensation and endosomal release, which can improve gene transfection efficiency. Compared with liposome-based method, cationic polymers are very stable, easy to handle and more resistant to serum in cell culture. The above advantages make gene transfection much easier and reproducible. E-Fect can be successfully used in both established cell lines and primary cells. Some cell lines have been successfully transfected with E-Fect as shown in Table 1. Transfection can be finished in half an hour. E-Fect is a serum resistant reagent; the transfection procedure is much simpler than traditional methods: E-Fect / DNA complex can be directly added into complete cell medium. With no need to change and wash cell medium, the 2-step hands-on transfection procedure can be completed in less than 30 minutes.

Table 1. Cell lines successfully transfected with E-Fect

Cell line	Description	Transfection efficiency
293T	Human cell line, derived from the Human Embryonic Kidney (HEK) 293 cell line	90-98%
A549	Human lung cancer cell line	
BHK-21	Hamster Kidney cells, for viral transfection studies	80-95%
C6/36	*Aedes albopictus* (for Mosquito cell line), commonly in arthropod-borne viruse studies)	
CHO-K1	Chinese hamster ovary cells	70-90%
DLBCL	Diffuse large B cell lymphoma	
ES-2	Human ovarian cancer cells	
HCT116	Human colon cancer cells	
HeLa	Human cervical cancer cell line	
HepG2	Human liver cancer cell line	75-90%
HMEC-1	Human microvascular endothelial cells	40-65%
HT-29	Human cancer colon cell line	
HuH-7	Human liver cancer cell line	75-95%
HUVEC	Human umbilical vein endothelial cells (Primary cells)	
K562	Human erythroleukemic (blood) cell line	

MDCK	Madin-Darby Canine (dog) kidney cell, commonly used in cell biology studies	65-80
PBMC	Human peripheral blood mononuclear cells	
S2	*Drosophila melanogaster* cell line	
SF9	*Spodoptera frugiperda* (insect) *cell line*	
SKNSH	Human neuroblastoma (brain)	25-60%
SP2/0	Mouse myeloma cell line	
THP-1	Human leukemic monocytic cell line	
U118 MG	Human brain glioma cells (glioblastoma)	25-40%
Vero	Monkey kidney epithelial cells	40-80%

Transfection protocol

For Adherent cells:

1. Cell seeding

To obtain optimal transfection efficiency with E-Fect, the cell density should be 70-90% confluent for adherent cell or 5×10^5 to 2×10^6 cells/mL for suspension cells. Make sure that these cells are viable and healthy. The cells were seeded at 18-24 hours before gene transfection. Table 2 shows the recommended number of cells to be seeded in different cell culture device.

Note: The medium should be refreshed 30 mins before transfection. Usually, culture media with serum does not affect transfection.

2. Preparation of complex

Guideline of plasmid DNA and E-Fect DNA Transfection Reagent amount and ratio refer to the following Table 3. DNA plasmid for transfection should be with high purity (A260/A280=1.8~1.9) to ensure efficient transfection mixture preparation.

(1) For 6 well plate: Dilute 1μg plasmid DNA in 50μl serum-free and antibiotic-free medium, mix well gently to create DNA Solution.

(2) Dilute 3μl E-Fect in 47μl medium, mix gently to create E-Fect Solution. Mix well gently.

(3) Add 50μl E-Fect Solution to 50μl DNA Solution and **mix with vortex** to create E-Fect / DNA complex.

Notes: The order of mixing two solutions is very important for gene transfection results. Do not reverse the order.

3. Incubate for 15-20 min at room temperature.

4. Add 100μl E-Fect / DNA complex gently into each cell culture dish/plate while gently swirling the plate.

The E-Fect / DNA complex could be removed after 6 hours and refilled with culture medium.

5. Incubate cells at 37°C in a CO2 incubator. The transfection efficiency of reporter gene (ex: GFP or luciferase) could be analyzed 24-48 hours after transfection.

Table 2. Recommended numbers of cells to be seeded in cell culture device

Culture dish/plate	Area of device (mm^2)	Cell number	Final volume of medium in cell culture
96 well plate	50	$1.5\text{-}5\times10^4$	100 µl
24 well plate	200	$8.0\times10^4\text{-}2.0\times10^5$	500 µl
12 well plate	401	$1.6\text{-}4.0\times10^5$	1.0 ml
6 well plate	962	$3.0\text{-}8.0\times10^5$	2.0 ml
35 mm dish	962	$3.0\text{-}8.0\times10^5$	2.0 ml
60 mm dish	2827	$1.0\text{-}2.5\times10^6$	6.0 ml
100 mm dish	7854	$2.5\text{-}6.4\times10^6$	10.0ml

For suspension cells:

At 1 hour after cell seeding (5×10^5 to 2×10^6 cells/mL), the E-Fect / DNA complex can be added into cells followed by transfection assay at 24-48 hours after transfection.

Table 3. Recommended transfection reagent and DNA in different cell culture device

Culture dish/plate	Medium volume	Plasmid/ medium	E-Fect DNA Transfection Reagent/ medium
96 well	100 µl	250 ng/ 10 µl	0.75 µl/ 10 µl
24 well	500 µl	500 ng/ 25 µl	1.5 µl/ 25 µl
12 well	700 µl	750 ng/ 35 µl	2.25 µl/ 35 µl
6 well	1 ml	1 µg/ 50 µl	3 µl/ 50 µl
35 mm	1 ml	1 µg/ 50 µl	3 µl/ 50 µl
6 cm	3 ml	2.5 µg/ 150µl	7.5 µl/ 150 µl
10 cm	6 ml	5 µg/ 300µl	15 µl/ 300 µl

Factors affect transfection efficiency

1. Amount of E-Fect and DNA used in gene transfection is dependent uon the size of cell culture device. Table 3 shows the recommended transfection reagent and DNA in different cell culture device. The user can optimize condition according to different experiment.

2. E-Fect is serum resistant and not affected by serum during transfection, so E-Fect / DNA complex can be directly added into complete cell medium. But the buffer for diluting E-Fect and DNA should be serum free, because E-Fect may bind the protein in serum before making E-Fect / DNA complex.

3. If the cell line is very sensitive, it is recommended that the transfection complex to be removed at 3-4 hour after adding complex followed by adding fresh medium containing serum.

4. Stable transfection

For stable transfection, 6-well plates or 35mm dishes are recommended to perform gene transfection according to the above protocol. The cells could be selected 24-48 hours after transfection.

Troubleshooting

Problems	Suggestions
Low transfection efficiency	1.Use optimal amount of plasmid. 2.Use high quality plasmid (OD260/280 >1.8). 3.The density and morphology of cell is optimal. 4. Optimize the E-Fect/DNA ratio (w/w from 5:1 to 3:1). 5. Set positive control, such as GFP gene and luciferase gene.
Cell toxicity	1. The healthiness of cell affect the cytotoxicity. 2. The cytotoxicity will increase, if the cell density is not optimal. 3. Reduce the incubation time for some sensitive cell lines. 4. Check gene product is toxic or not. 5. Make sure the plasmid is free of endotoxin.!

Notice: This reagent is for research use only.