

Introduction

The delivery of foreign DNA into eukaryotic cells is one of the most common molecular biology techniques to study biological mechanisms. However, unlike transformed cell lines, the efficient transfection of primary cells can be a problem. EpiFectagen is a cationic polymer-based transfection system specifically designed and optimized for efficient transfection of primary epithelial cells. Transfection with EpiFectagen can be carried out in the presence of antibiotics and serum. Instead of normal two-day transfection, an optimized one-day transfection procedure can be performed for time-saving and highly reproducible transfection. 1.5 ml of EpiFectagen reagent is sufficient for up to 100 transfections per well in 96-well plate.

Storage/Handling

Upon receipt, aliquot and store EpiFectagen reagent A at -20°C, avoid repeated freezing/thawing cycles. Once thawed, store EpiFectagen reagent A at 4°C and use in a month. EpiFectagen reagent B can be kept at 4°C.

Quality Control

Each lot of EpiFectagen is performance tested by transfecting Human Renal Proximal Tubular Epithelial Cells (HRPTEpiCs, Cat. No. 4100, ScienCell[™]) with Promega[®] pSV-bata-Galactosidase control vector. Gene expression is assayed by X-gal staining 24 hours post transfection. Typically, 30-60% transfection efficiency can be achieved (Figure 1).

Procedures for Transfecting Adherent Cells in 96-well Plate*

A. Preparation of cells

1. On the day of transfection, coat 96-well plate with poly-L-lysine at 2 µg/cm². Incubate at 37°C for 2-4 hours. Rinse the poly-L-lysine coated wells with sterile deionized H₂O twice before seeding of cells. The pre-coating of poly-L-lysine ensures good and even epithelial cell adhesion.
2. Select a flask of epithelial cells with 60-80% confluency, harvest and dilute cells in Epithelial Culture Medium to give a final concentration of $\sim 1.1 \times 10^5$ cells/ml.

B. Transfection complex formation

1. Prepare plasmid DNA in sterile deionized H₂O to give a final concentration of 1 µg/µl. To achieve successful transfection, high quality DNA with OD₂₆₀/OD₂₈₀ of 1.8 or greater is recommended.
2. For each well, add 0.5 µl plasmid DNA, 10.5 µl sterile deionized H₂O and 2 µl EpiFectagen reagent B into a 1.5 ml sterile plastic tube. Vortex gently and spin down briefly. Then add 7 µl EpiFectagen reagent A to make the total volume of the transfection mixture to be 20 µl, vortex for 5 seconds and spin down. Incubate at room temperature for 20-30 min.

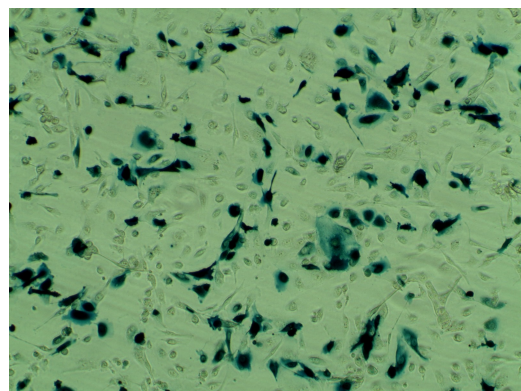


Figure 1. HRPTEpiCs expressing β-galactosidase after transfection using EpiFectagen.

C. Incubation of cells with transfection mixture

1. Plate 180 μ l of cell suspension ($\sim 1.1 \times 10^5$ cells/ml) in each well to give $\sim 2 \times 10^4$ cells per well.
2. Add 20 μ l of transfection mixture to each well. Mix by gently rocking the plate side-to-side.
3. Culture the cells for ~ 24 hours under standard conditions. Or perform a medium change after 4-6 hours' incubation with transfection mixtures, replace with 200 μ l fresh culture medium, and culture for additional 16-18 hours. Generally longer incubation time with transfection mixture results in increased transfection efficiency and decreased cell viability.
4. Harvest cells 24 hours post transfection and assay for gene expression.

* The amounts of cells and various transfection reagents mentioned in the instruction are recommended for performing transfection in 96-well plate. For transfection in larger size wells, the amounts of epithelial cells and transfection reagents (DNA, sterile deionized H₂O and EpiFectagen reagents A&B) should be scaled up according to the surface area of the wells (Table 1).

Culture Vessel	Growth Area (cm ² /well)	# of cells	1 μ g/ μ l DNA stock (μ l)	Sterile DI H ₂ O (μ l)	EpiFectagen reagent B (μ l)	EpiFectagen reagent A (μ l)	EpiCM (μ l)
96-well plate	0.35	20,000	0.5	10.5	2	7	180
48-well plate	0.8	45,000	1.1	24	4.6	15.9	411
24well plate	2.0	115,000	2.9	60	11.6	40	1029
12-well plate	4.0	230,000	5.7	120	23	81	2057
6-well plate	9.6	550,000	13.7	288	55	193	4937

Table 1. Recommended quantities of epithelial cells and EpiFectagen reagents per well.