Rev. 1

ScienCell™
Research Laboratories

EpiFectagen II (EpiF)

Catalog #0973

250 transfections in 96-well plate

Product Description

The delivery of foreign DNA into eukaryotic cells is one of the most common molecular biology techniques used to study biological mechanisms. However, unlike transformed cell lines, efficient transfection of primary cells can be challenging. EpiFectagen II is a cationic polymer-based transfection system specifically designed and optimized for serum-free cultured primary epithelial cells, including Human Esophageal, Bronchial, Tracheal, Small Airway, Prostate, Corneal, Ovarian Surface, and Mammary Epithelial Cells. Transfection can be carried out in the presence of antibiotics and features a simple protocol compatible with multiple culture formats (6-, 12-, 24-, and 96-well plates). An optimized one-day transfection procedure can be used instead of a conventional two-day workflow, providing time-saving and highly reproducible results.

Kit Components

Cat#	Component	Quantity	Storage
0973a	EpiFectagen Reagent A	1 vial	-20°C
0973b	EpiFectagen Reagent B	1 vial	-20°C

Materials Supplied by User (Not provided)

- Primary epithelial cells
- Serum-free medium
- DNA plasmid, siRNA.
- Cell culture plates (6-, 12-, 24-, or 96-well)
- Standard tissue culture equipment (pipettes, incubator, etc.)
- pSV-β-Galactosidase control vector
- Poly-L-lysine (PLL)
- Deionized H_2O

Product Use

EpiF is for research use only and is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Quality Control

EpiFectagen performance was validated using Human Prostate Epithelial Cells (HPEpiCs, Cat. No. 4410, ScienCell™) transfected with Promega® pSV-β-Galactosidase control vector. Typically, ~10% transfection efficiency was achieved 24 hours post-transfection, as measured by X-Gal staining (Figure 1).

Shipping and Storage
Upon receipt, aliquot and store at -20°C, avoid repeated freezing/thawing cycles. Product is shipped on dry ice.

Procedures for Transfecting Adherent Cells (Example for 96-well Plate)

1. **Preparation of Coverslips and Cell Seeding**
   - **1.1.** Coat 96-well plates containing sterile cover glasses with poly-L-lysine (PLL) at a final concentration of 2 µg/cm².
   - **1.2.** Incubate at 37°C for 2–4 hours, then rinse twice with sterile deionized H_2O and allow the surface to dry inside the biosafety cabinet, before seeding of cells.
   - **1.3.** Harvest epithelial at ~60–80% confluency and dilute cells in Culture Medium to give a final concentration of ~1.1 x 10^5 cells/mL.
     Note: The pre-coating of poly-l-lysine ensures good and even epithelial adhesion.

2. **Preparation of DNA–EpiFectagen II Complexes**
   - **2.1.** Prepare plasmid DNA stock at 1 µg/µL in sterile deionized H_2O.
   - **2.2.** For each well of a 96-well plate:
   - **2.3.** Add 0.5 µL plasmid DNA, 12 µL sterile deionized H_2O, and 2 µL EpiFectagen II Reagent B to a sterile microtube.
   - **2.4.** Vortex gently and spin down briefly.
   - **2.5.** Add 5.5 µL EpiFectagen II Reagent A, to make the total volume of the transfection mixture to be 20 µL.
   - **2.6.** Vortex for 5 seconds, and spin down again.
   - **2.7.** Incubate the transfection mixture for 20–30 minutes at room temperature to allow complex formation.
     Note: Complex formation in sterile deionized H_2O *provides optimal complex and reproducible efficiency. Serum-free medium can also be used.*

3. **Transfection**
   - **3.1.** Plate 180 µl of cell suspension (~1.1×10^5 cells/ml) in each well to give ~2×10^4 cells per well.
   - **3.2.** Add the transfection mixture (total 20 µL) dropwise to each well.
   - **3.3.** Gently rock the plate side-to-side to ensure even distribution of complexes over the cells.
   - **3.4.** Incubate under standard conditions (37°C, 5% CO_2) for 24 hours.
     Optional: Medium can be replaced after 4–6 hours to minimize cytotoxicity. Longer incubation increases efficiency but reduces viability.

4. **Post-Transfection Analysis**
   - **4.1.** Harvest cells 24 hours post transfection and assay for gene expression.

   Note: Cell and reagent amounts listed are recommended for 96-well plates. For larger wells, scale up of epithelial and transfection reagents (DNA, sterile deionized H_2O, EpiFectagen II A&B) proportionally according to well surface area (Table 1).

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

Culture Vessel	Growth Area (cm^2/well)	# of Cells	1 µg/µL DNA stock (µL)	Sterile DI H_2O (µl)	EpiFectagen II Reagent B (µL)	EpiFectagen II Reagent A (µL)	Medium (µL)
96-well plate	0.35	20,000	0.5	12	2	5	180
48-well plate	0.8	45,000	1.1	27	4.6	12.6	411
24-well plate	2.0	115,000	2.9	69	11.6	31	1029
12-well plate	4.0	230,000	5.7	137	23	63	2057
6-well plate	9.6	550,000	13.7	329	55	151	4937

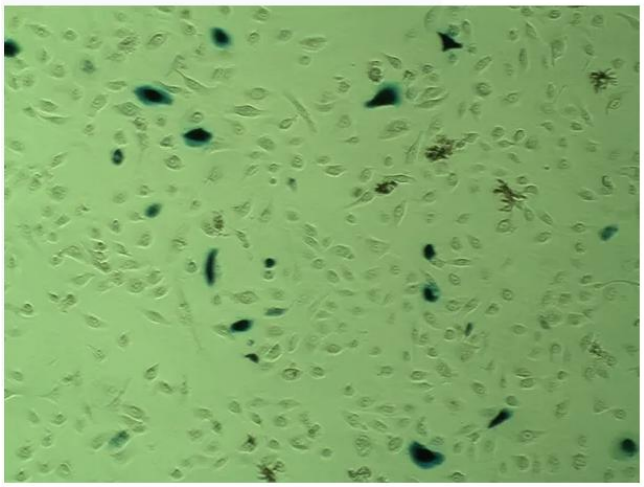

Figure 1: HPEpiCs expressing β-galactosidase after transfection using o EpiFectagen II.

Troubleshooting Table

Problem	What it means	Possible Cause	Solution
Low efficiency	Few transfected cells (<50% X-gal–positive cells)	Incorrect DNA: reagent ratio	Increase Reagent B by 20%; test ±20% of DNA amount from Table 1
High cytotoxicity	>20-30% cell death post-transfection	Reagent A overdose or no serum	Reduce Reagent A by 10%; replace medium at 4h
Aggregates/precipitates	Clumps in mix or uneven cell coverage	Excessive vertexing of reagents	Pipette gently; use fresh DI H_2O
Slow/weak expression	Low signal even at 48h	Too much media volume	Reduce well media to 400 µL before complex