Rev. 1

ScienCell™
Research Laboratories

KeraFectagen (KeraF)

Catalog #0963

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. KeraFectagen is a cationic polymer-based transfection system specifically designed and optimized for primary keratinocytes. It can be used in the presence of serum and antibiotics and features a simple protocol compatible with both serum-containing and serum-free conditions. The reagent supports multiple culture formats (6-, 12-, 24-, and 96-well plates), and an optimized one-day transfection procedure can be used instead of a conventional two-day workflow, providing time-saving and highly reproducible transfection.

Kit Components

Cat#	Component	Quantity	Storage
0963a	KeraFectagen Reagent A	1 vial	-20°C
0963b	KeraFectagen Reagent B	1 vial	-20°C

Materials Supplied by User (Not provided)

- Primary keratinocytes
- Complete growth medium suitable for the chosen cell
- 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

KeraF 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

KeraFectagen performance was validated using Human Epidermal Keratinocytes (HEKs, Cat. No. 2100, ScienCell™) transfected with Promega® pSV-β-Galactosidase control vector. Typically, ~40-60% 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 keratinocytes at ~60–80% confluency and dilute cells in Keratinocyte Culture Medium to give a final concentration of ~11.0 x 10^4 cells/mL.
     Note: The pre-coating of poly-l-lysine ensures good and even keratinocytes adhesion.

2. **Preparation of DNA–KeraFectagen 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, 12.5 µL sterile deionized H_2O, and 2 µL KeraFectagen Reagent B to a sterile microtube.
   - **2.4.** Vortex gently and spin down briefly.
   - **2.5.** Add 5 µL KeraFectagen 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 (~11.0×10^4 cells/ml) in each well to give ~2.0×10^4 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 keratinocytes and transfection reagents (DNA, sterile deionized H_2O*, KeraFectagen A&B) proportionally according to well surface area (Table 1).*

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

Culture Vessel	Growth Area (cm^2/well)	# of Cells	1 µg/µL DNA stock (µL)	Sterile DI H_2O (µl)	KeraFectagen Reagent B (µL)	KeraFectagen Reagent A (µL)	Medium (µL)
96-well plate	0.35	20,000	0.5	12.5	2	5	180
48-well plate	0.8	45,000	1.1	29	4.6	11.4	411
24-well plate	2.0	115,000	2.9	71	11.4	29	1029
12-well plate	4.0	230,000	5.7	143	23	57	2057
6-well plate	9.6	550,000	13.7	343	55	137	4937

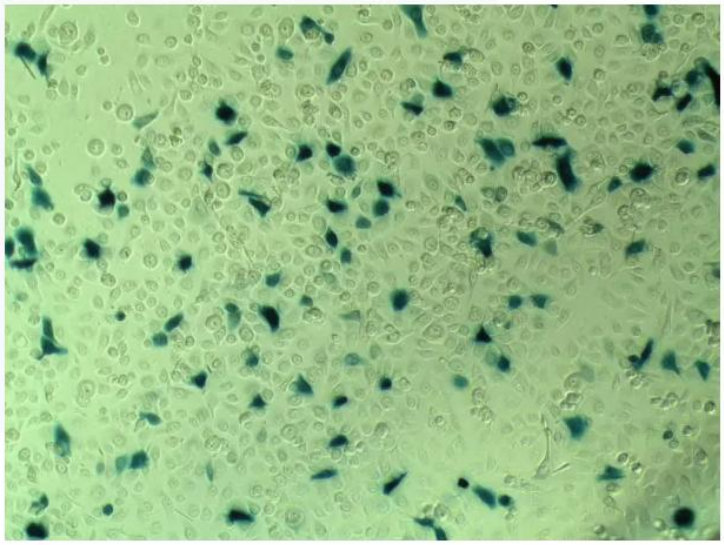

Figure 1: HEKs expressing β-galactosidase after transfection using o KeraFectagen.

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