KeraFectagen

Cat. No. 0963, 250 transfections in 96-well plate

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. KeraFectagen is a cationic polymer-based transfection system specifically designed and optimized for efficient transfection of primary keratinocytes. Transfection with KeraFectagen 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. 1.25 ml of KeraFectagen reagent is sufficient for up to 250 transfections per well in 96-well plate.

Storage/Handling

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

Quality Control

Each lot of KeraFectagen is performance tested by transfecting Human Epidermal Keratinocytes (HEKs, Cat. No. 2100, ScienCell™) with Promega® ρSV-bata-Galactosidase control vector. Gene expression is assayed by X-gal staining 24 hours post transfection. Typically, 40-60% 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/cm^2. Incubate at 37°C for 2-4 hours. Rinse the poly-l-lysine coated wells with sterile deionized H_2O twice before seeding of cells. The pre-coating of poly-l-lysine ensures good and even keratinocytes adhesion.
2. Select a flask of keratinocytes with 60-80% confluency, harvest and dilute cells in Keratinocyte Medium to give a final concentration of ~11.0×10^4 cells/ml.

B. Transfection complex formation

1. Prepare plasmid DNA in sterile deionized H_2O to give a final concentration of 1 µg/µl. To achieve successful transfection, high quality DNA with OD_{260}/OD_{280} of 1.8 or greater is recommended.
2. For each well, add 0.5 µl plasmid DNA, 12.5 µl sterile deionized H_2O and 2 µl KeraFectagen reagent B into a 1.5 ml sterile plastic tube. Vortex gently and spin down briefly. Then add 5 µl KeraFectagen 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.

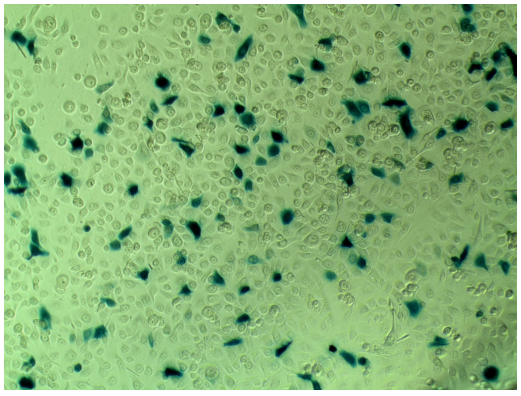

Figure 1. HEKs expressing β-galactosidase after transfection using KeraFectagen.

C. Incubation of cells with transfection mixture

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.
2. Add 20 µl 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 µl 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 keratinocytes and transfection reagents (DNA, sterile deionized H_2O and KeraFectagen reagents A&B) should be scaled up according to the surface area of the wells (Table 1).

Table 1. Recommended quantities of keratinocytes 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)	KM (µl)
96-well plate	0.35	20,000	0.5	12.5	2	5	180
48-well plate	0.8	46,000	1.1	29	4.6	11.4	411
24well 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