Transfection of primary adult human keratinocytes using K2 Transfection System

Materials:

- KGM-Gold™ media (Lonza)
- 12-well plate (BD bioscience)
- Sterile Eppendorf tubes
- Trypsin solution
- PBS
- Plasmid DNA: pE-GFP-C1 (Clontech)
- Transfection reagents:
  K2 transfection reagent
  K2 multiplier

Protocol

1. Freshly isolated primary human keratinocytes were seeded on 12-well plate (BD bioscience) in density $5x10^4/cm^2$. Cells were grown in 1ml KGM-Gold™ media (Lonza) with 1% penicillin-streptomycin to app. 70% of confluence (app. 7 days).
2. K2 multiplier (10µl) was added to cells 2h prior transfection. Transfection mixtures were prepared 20 minutes prior transfection according to Table I and following protocol:

Table I.

DNA:K2 ratio- **1:2**
Plate: **12-well plate**
Amount of medium per well: **1ml**
K2 multiplier: 10µl, 2h incubation

DNA [µg]	KGM-gold [µl]	K2 [µl]	KGM-gold [µl]
0,3	50	0,6	50
0,5	50	1	50
0,8	50	1,6	50

The indicated amount of K2 transfection reagent (See Table I) was added to 50 µl of serum-free cell culture medium (KGM-gold) in a 1.5 ml Eppendorf centrifuge tube. In a separate tube indicated amount of the plasmid DNA (pE-GFP-C1) was mixed with 50 µl of serum-free medium. The media containing K2 and DNA were combined and mixed by pipetting up and down 5 times. The tubes were incubated at room temperature for 20 min for lipid-DNA complexes to form. At the end of the incubation time, the lipid-DNA complex mixtures (100 µl per well) were pipetted onto the cells, mixed by tilting the plates a few times and thereafter incubated at 37°C incubator under 5% CO2. Medium was changed after 24h and cells were analyzed under fluorescent microscope.

Results

Best efficiency (40%) was obtained with 0,8µg DNA and 1,6µg K2 per well (Figure 1).

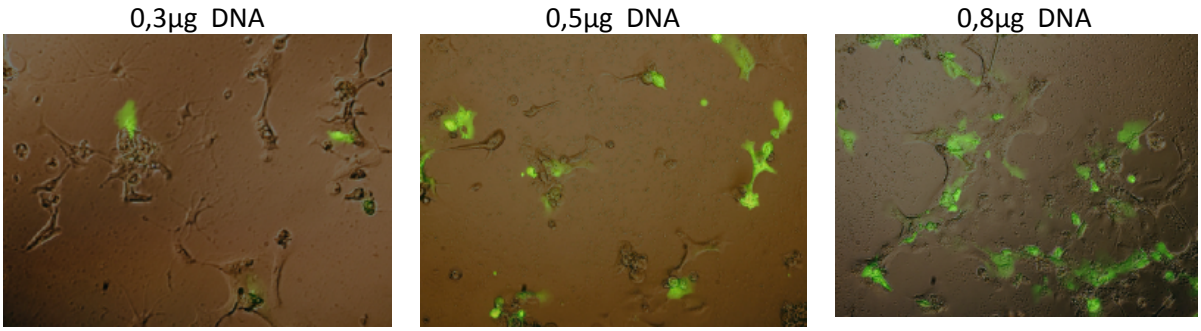

Figure 1. GFP expression in primary human keratinocytes after transfection with different DNA amount.