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DNA-transfection of immortalized human kidney epithelial cells (IHKE1) using "Biontex K2® Transfection System".

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Materials and Methods

Cell culture

IHKE-1 cells were cultivated in 35 mm Ø cell culture dishes on glass cover slips or 100 mm Ø cell culture dishes [1]. The cells were a kind gift of Thomas Weide, UKM, Muenster, Germany. The culture medium was DMEM/Ham's F12 supplemented with 1% FBS, 15 mM HEPES (pH 7.2), 44 mM $NaHCO_3$, 1 mM sodiumpyruvate, 4.5 mM L-glutamine, 36 ng/ml hydrocortisol, 10 ng/ml EGF, 5 mg/ml insulin, 5 mg/ml transferrin and 5 ng/ml sodium selenite. The amount of medium for 100 mm and 35 mm dishes were 10 ml and 1.5 ml, respectively. Transfection in 35 mm dishes was carried out after 1×10^5 cells were cultured for 24 hours. Transfection in 100 mm dishes was performed when cells had reached a confluency of 70-90%. Cells were treated with K2® Multiplier 2 hours before DNA transfection (35 mm Ø dishes: 2.5 µl; 100 mm Ø dishes: 20 µl).

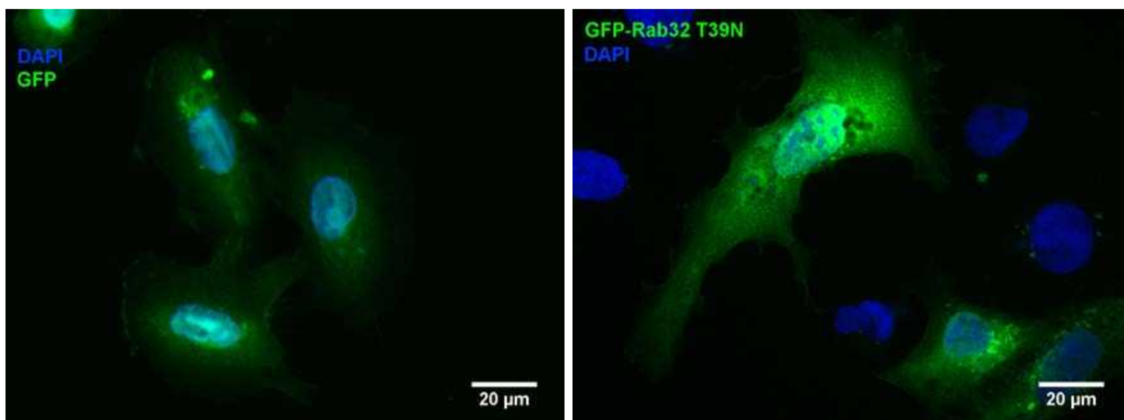
K2® Transfection

The protocol is given for the 35 mm dishes (100 mm Ø dish protocol in brackets). 0.3 µg (2.4 µg) of plasmid DNA was mixed with 15 µl (120 µl) of DMEM/Ham's F12. In a second tube, 1.2 µl (9.8 µl) of K2® were mixed with 15 µl (120 µl) of DMEM/Ham's F12 by pipetting 1 x up and down. The DNA solution was then added to the K2® solution and mixed by 1 x pipetting up and down. After incubation for 20 minutes at room temperature the transfection mix was added to the cells dropwise while gently shaking the cell culture dish. Transfections were incubated at 37^0C and 8% CO_2 for 24 hours. Transfection efficiency was estimated by fluorescence microscopy and/or by immunoblotting for proteins encoded by the transfected plasmids.

Results

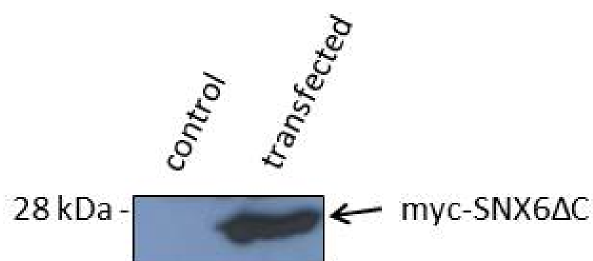
Fluorescence microscopy

Fluorescence microscopy shows successful transfection of IHKE-1 cells with pEGFP-C1 grown on glass cover slips in 35 mm Ø cell culture dishes. The cells show a healthy morphology, the transfection rate was good (≥ 50%). The same result was obtained for IHKE-1 cells grown in 100 mm Ø culture dishes (data not shown). Transfecting IHKE-1 cells with plasmids encoding GFP fusion proteins revealed good transfection efficiencies as well.



Immunoblot of myc-tagged protein

For the immunoblot DNA encoding a myc-tagged protein was transfected into IHKE-1 cells using the K2® transfection system with the 100 mm Ø cell culture dish protocol. The blot against the myc-tag shows a band of the expected size (~25 kDa). A control plasmid expressing just the GFP peptide was used to determine the transfection efficiency (≥ 50%) by microscopic analyses (data not shown).



Literature

1 Tveito, G., Hansteen, I. L., Dalen, H. & Haugen, A. Immortalization of normal human kidney epithelial cells by nickel(II). *Cancer Res* **49**, 1829-1835 (1989).