DNA-transfection of Madin Darby Canin Kidney cells (MDCK II) using the „Biontex K2®Transfection System"

Dipl.-Biol. Caroline Pasquay, Laboratory of Molecular Ophthalmology, University Eye hospital, Justus Liebig University Giessen, Friedrichstrasse 18, 35392 Giessen, Germany

Materials and Methods

Cell culture

MDCK II cells were cultured with frames (ø 11 mm / cavity) on SuperFrost®microscopy slides (Langenbrink) or on transwell filter inserts (Greiner) on 6-well plates (Greiner) in Dulbecco's modified eagle medium (PAN) supplemented with 10%fetal calf serum (PAN), 5% L-glutamine and 5% Pen/Strep (Biochrome). Transfection was performed when cells had reached a confluency of 60-70%.

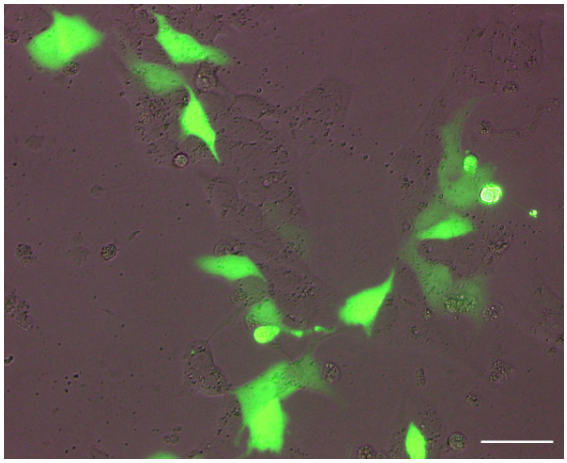
Cell transfection

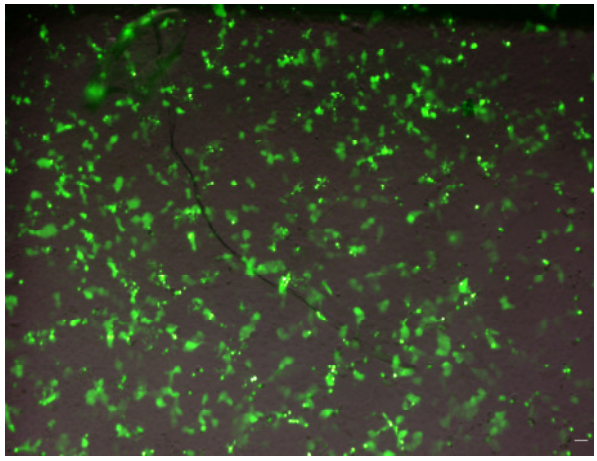
Cells were treated with K2® Multiplier 2 hours before transfection. For this K2® Multiplier (please find accurate reagent amounts in the table below) was dripped slowly onto the medium and mixed by gently swaying. K2® Transfection Reagent was mixed with unsupplemented Dulbecco's modified eagle medium (DMEM) (PAN) and incubated at room temperature during preparation of the DNA. Plasmid DNA encoding eGFP was mixed with unsupplemented DMEM. DNA solution was added to the solution containing K2® Transfection Reagent (sequence is very important!) and mixed by pipetting the solution up and down once, followed by 20 minutes incubation at room temperature. Transfection solution was applied to the cells by slow dropwise addition to the medium followed by gently swaying to achieve mixing. Transfections were incubated at 37°C and 5% CO_2 for 24 hours. Transfection efficiency was estimated by fluorescence microscopy.

Well size	DMEM	K2® Multiplier	K2® Transfection Reagent	Unsupplemented DMEM	DNA
11 mm	250 µl	5 µl	1,2 µl	15 µl	300 ng
35 mm	800 µl	45 µl	11 µl	50 µl	2400 ng

Results

Transient transfection of eGFP in MDCK II cells grown on Superfrost microscopy slides (left) and on transwell filters on a 6-well plate (right)





Scale bar: 50 µm

Aggregates on the right photo are due to the filters.

Conclusions

Fluorescence microscopy showed successful transfection of eGFP with high transfection rates. The MDCK II cells show a perfectly healthy morphology and a healthy phenotype. The Data revealed high transfection rates (60-70 % without further optimization) without cytotoxic effects of the transfection system.

In contrast to other transfection systems two properties of the K2® Transfection system are advantageous.

1. Efficiency.
   MDCK II cells prefer lower confluency rates (40 – 50%) at transfection. With the K2® Transfection system these cells could be transfected at higher confluence. The low amounts of DNA and transfection reagent avoid cytotoxic effects to the cells. The transfection rate was at about 60 %.

2. Physiology
   MDCK II cells appear perfectly healthy after DNA transfection.