# Co-transfection of ARPE-19 Cells by Using Biontex K2® Transfection System

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This experiment was carried out to test transfection efficiency of the K2 transfection kit.

## Cell culture

ARPE-19 cells were cultured in 24-well plates (Thermo-Scientific) in antibiotic-free DMEM:F12 medium with 10% fetal bovine serum (Life Technologies). Cells were seeded at a density of 1 x $10^5$ cells/well.

## Transfection

Transfection was carried out when cells reached 90% confluency, normally one day after seeding.

Five µl K2® Multiplier was added drop-wise into a well containing ARPE19 cells and 500 µl DMEM-F12-FBS, and mixed by gently swaying the plates. No multiplier was added to the control well. Cells were incubated in the K2® Multiplier mixture (or normal medium for the control) for 2 hours.

At about 20 min before transfection, prepare the following amount of transfection mixture for each well. First, 0.6-µg plasmid DNA and 1.2-µl K2® Transfection reagent were each added to 30µl serum-free DMEM:F12 medium in separate tubes and mixed by gently pipetting 1-2 times. Our DNA plasmids in the current case were mCherry-C1 vector (0.15µg) (Clonetech) and pXJ40-Flag-Juxtanodin (JN) (0.45µg). Then the DNA-medium mixture was added to the transfection reagent-medium mixture, and mixed by gently pipetting 1-2 times. This DNA-lipid complex solution was incubated at room temperature for 15 minutes before being added drop-wise to the wells of cells containing DMEM-F12-FBS (500µl) and K2 multiplier (0 µl or 5 µl). After mixing by gentle swaying, the plate was put into 37°C, 5% $CO_2$ incubator. The culture medium was changed 24 hrs after to DMEM:F12 medium (Life Technologies) with 10% fetal bovine serum. Transfection efficiency could be checked by viewing mCherry signal under fluorescent microscope (Nikon Eclipse TE2000-S). Cells were then cultured for an additional 2 hours.

## Immunofluorescence staining

To further confirm the transfection efficiency, immunofluorescence staining was conducted. The cells were washed briefly in PBS (phosphate buffered saline, 0.01M, pH7.4), fixed in 3% paraformaldehyde for 30 min, washed again in PBS, and pre-incubated in PBS-T-NGS (0.3% Triton X-100, 6% Normal Goat Serum). They were then incubated in mouse anti-FLAG tag antibody (1:1000 in PBS-T-NGS, Sigma) solution overnight. The secondary antibody Alexa Fluor 488 Goat anti-mouse IgG (1:400 in TBS-

T-NGS, Life Technologies) was used to detect bound primary antibody. After coversliping, the cells were viewed and photographed at 10x magnification under an inverted microscope attached with a digital camera (Nikon).

**Results and Discussion**

Cell survival appears to be at around 80% while a co-transfection efficiency of 15-20% was observed. This efficiency might increase considerably if transfection of a single plasmid instead of co-transfection of 2 plasmids, were carried out. The K2® Multiplier does not seem to significantly increase cell viability or transfection efficiency in ARPE-19 cells.

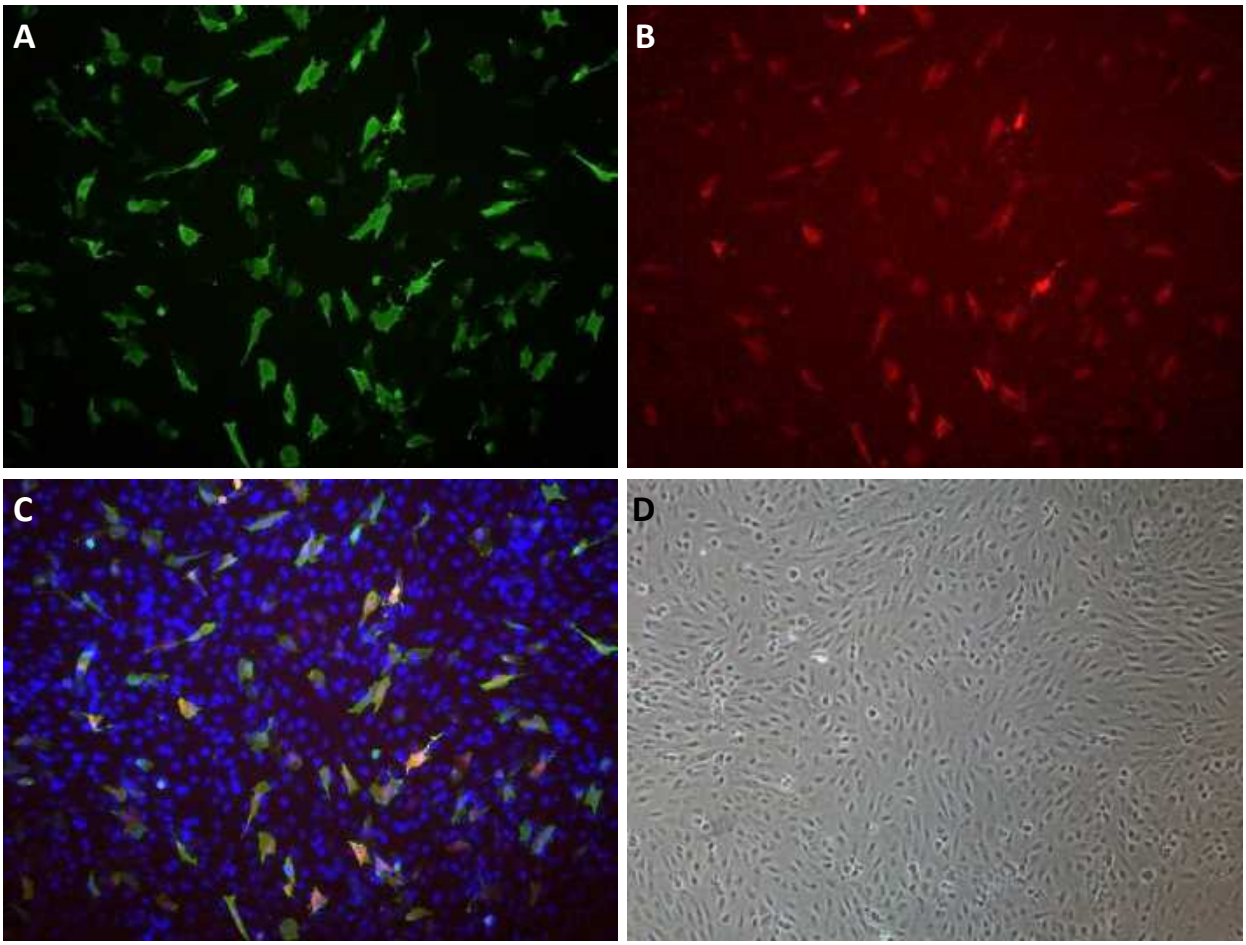


*Figure 1.* Co-transfection of ARPE-19 cells by using K2 transfection kit (but no addition of the K2 multiplier). Juxtanodin-transfected cells were immuno-stained green (***A***). mCherry is red (***B***). Panel ***C*** shows the merge of ***A*** and ***B***. Cell nuclei were stained by DAPI (blue) in ***C***. Panel ***D*** shows phase-contrast image of the field. Co-transfection efficiency in this case is around 18%.