Transfection of polarized Caco-2 cells with the K2 Transfection System

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Human colon adenocarcinoma Caco-2 cells are a well-established system to study cell polarity and trafficking. These studies may include the over expression of certain molecules which can be attained via transfection. However, CaCo-2 cells commonly display lower transfection efficiency as compared to other cell lines. In this assay we compare the transfection efficiency of Biontex reagents against the commonly used Lipofectamine 2000.

Materials and Methods

Cell culture

Colon adenocarcinoma CACO-2 cells were obtained from the American Type Culture Collection (Manassas, VA). They were maintained in Dulbecco's modified Eagle's medium-F12 supplemented with 10% fetal bovine serum and 1mM sodium pyruvate (Life Technologies, Carlsbad, CA) at 5 % CO2 and 37^{o}C. The cell stocks were kept in 25 cm^{2} tissue culture flasks and were collected after dissociation with 0.25 % trypsin, 2 mM EDTA for fifteen minutes. The cells were seeded on 6.5 mm Transwell-Clear ^{TM} filters (Corning Costar Corp. Cambridge MA) at high density (~5x10^{4} cells/cm^{2}) to obtain confluency in 2–3 days.

Cell transfection

Caco-2 cells at 75% confluence were transiently transfected with a vector tagged with GFP, using Lipofectamine 2000 (Life Technologies) or K2 Transfection System (Biontex , Munich, Germany) following manufacturer instructions. The growth area of the filters is 0.33cm^{2}. Lipofectamine 2000 was used at a volume of 0.34 µl and 0.1 µg of DNA (optimized quantities). Biontex reagents were used as described below; CaCo-2 cells were incubated with 2.0µl of K2 Multiplier for two hours, and then transfected with solutions containing 0.4µl of K2 Transfection reagent and 0.25µg of DNA. Each experiment was done in triplicate.

Fluorescence Microscopy

CaCo-2 cells grown to confluence were processed for fluorescence microscopy forty-eight hours after transient transfection. The cells were fixed with 4% paraformaldehyde for twenty minutes at room temperature. After rinsing, the cell monolayers were stained with DAPI and transferred to slides using Prolong anti-fade mounting reagent (Molecular Probes, Life Technologies). Fluorescent images were obtained using an EVOS fluorescent microscope (AMG, Bothell, WA). The images were collected using the EVOS software.

Results

CaCo-2 cells transfected with the K2 Transfection System appear to have higher GFP expression as compared to those transfected with Lipofectamine 2000.

CaCo-2 cells transfected with K2 Transfection System (lower panels) display a higher number of GFP expressing cells (green channel) as compared to cells transfected with

Lipofectamine 2000 (upper panels). This is also evident in the panels displaying both, GFP and DAPI (merge).

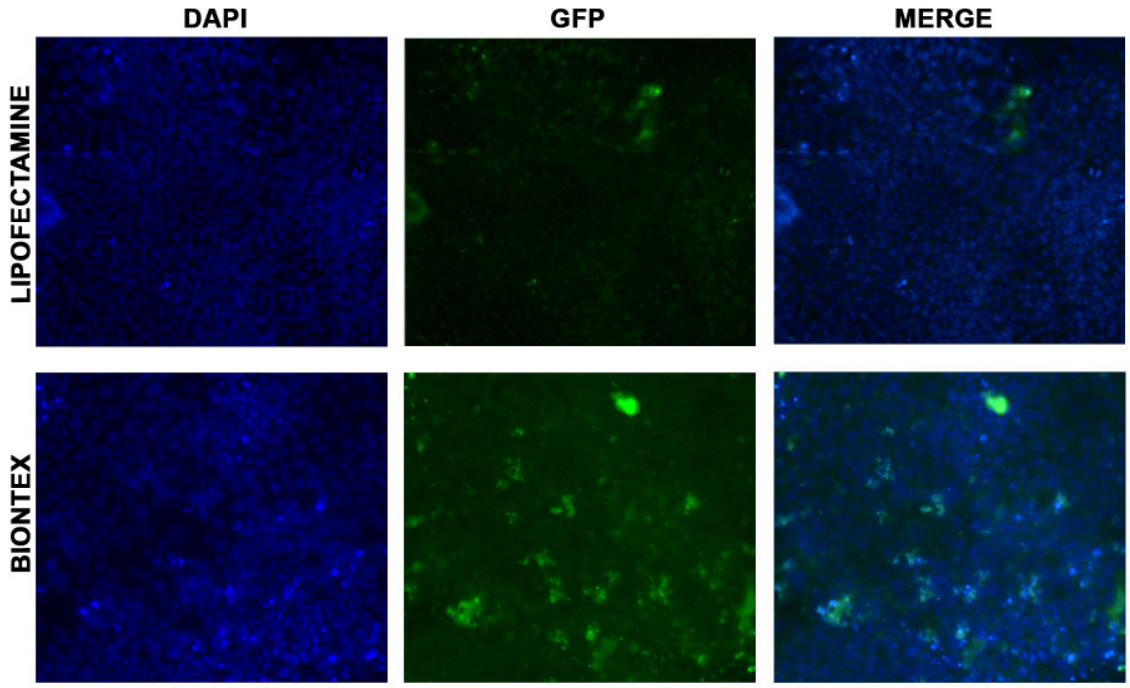

Figure legend
Fluorescence microscopy of GFP-expressing CaCo-2 cells. Representative images selected from at least eight fields, of CaCo-2 monolayers transfected with a GFP-tagged vector using Lipofectamine 2000 and K2 Transfection System are shown in the upper and lower panels, respectively. The presence of GFP-expressing cells (green channel) was confirmed by labeling the cells with DAPI (blue channel).