K2® Transfection System Technical Note

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Materials: Plasmid pCMV-eGFP
Sterile 24-well tissue culture plates
75 ml cell culture flasks
Sterile Eppendorf tubes
Trypsin solution
DMEM (PAA) + 10 % fetal calf serum
Opti-MEM®
HT-29 cells

Transfection reagents (bring to room temperature before use):
K2® Transfection Reagent
K2® Multiplier

Transfection of HT-29 cells:
HT-29 cells were grown in a 75 ml cell culture flask in DMEM with 10% serum without antibiotics to near confluency and trypsinated thereafter. 24 hours before transfection, HT29 cells were seeded in 500µl cell culture medium in 24-well plates at a density of $1x10^5$ cells per well. By the time of transfection, the cells were covering about 80% of the plate surface (80% optical confluency).

10 µl of K2® Multiplier was added to each well and the plate was returned to the incubator.

The transfection mix was prepared after 1 ½ hours of incubation with K2® Multiplier. 0.6 µg plasmid DNA was added to 30 µl Opti-MEM® in a 1.5 ml Eppendorf centrifuge tube. In a second tube, 2.4 µl K2® Transfection Reagent was added to 30 µl of Opti-MEM®. Both tubes were mixed by gentle tapping the tube. The solutions of the tubes were combined and mixed as described above. The tube was allowed to stand at room temperature for 20 min. After incubation the solution (60µl/well) was added dropwise to the cells. The transfection mix was distributed in the well by gently shaking the plate horizontally. The cells were placed back to the incubator. After 24 hours the transfection efficiency was monitored by fluorescence microscopy (Figure).

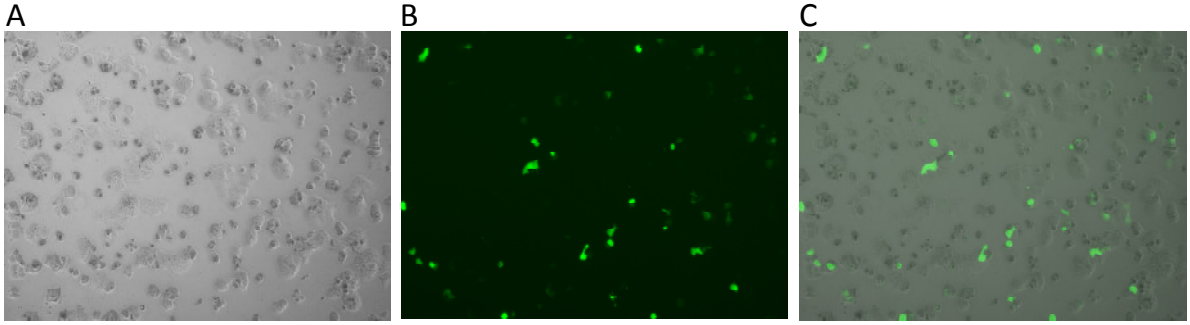

Figure: HT-29 cells were transfected with pCMV-eGFP using K2 Transfection Reagent as described above. Phage contrast image of transfected HT-29 cells (A), fluorescent image (B), and the overlay revealed the percentage of successfully transfected cells to be about 40%.