Application Note

BRAND*plates*® cellGrade™ plus surface minimizes loss of transfected cells during luminescence assays

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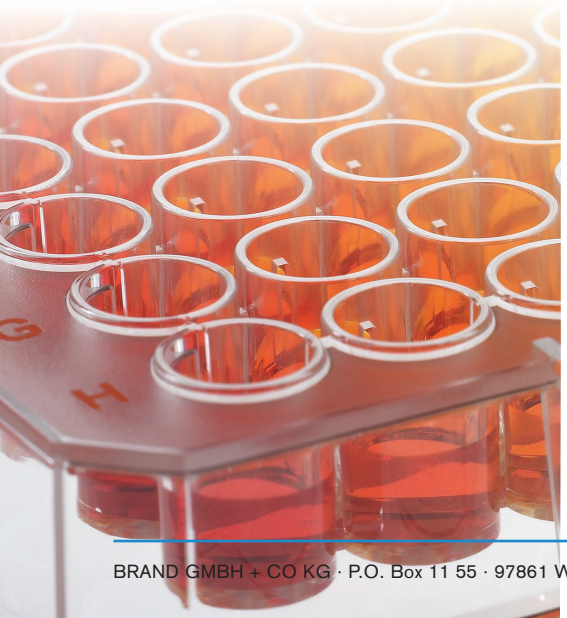
Introduction

Cell attachment is considered to be critical for many cell culture applications since it triggers for instance differentiation, metabolic activity and gene expression (Chang et al. 2012). Medium changes and washing steps especially in automated culture processing or high content screenings (HCS) can cause remarkable cell loss. To improve cell attachment to plastic surfaces two different methods are used. First, surfaces newly coated with extra cellular matrix proteins or peptides closely resemble the natural environment are known to be very effective in retaining cells during washing steps. Second, plasma induced polystyrene modifications (TC-treatements) are long lasting and the surface quality does not significantly decline over the first three years when stored properly (Jokinen et al., 2012).
Here we tested the BRAND*plates*® cellGrade™ plus surface to comparable TC-treated microplates from different well known manufacturers regarding their abi-lity to support cell proliferation and attachment after transfection. In this experiment success of transfection and cell retention during washing steps is followed by measuring luminescence signal intensity generated by luciferase transfected cells.



Material & Methods

25.000 HEK293.EBNA cells were seeded in DMEM (high glucose) supplemented with 10% FCS, 1x Penicillin/Streptomycin on white 96-well microplates with F-bottom. One day after seeding cells were transiently transfected with firefly and *Renilla* luciferase in-frame encoding plasmid using linear polyethylenimine at 40 kDa (PMID: 17084092). After an incubation time of 24 h, wells were washed 0-3 times with 200 µl PBS at 37 °C using an electronic 12-channel pipette at lowest down speed in order not to disturb the cell monolayer. Luciferase activity was determined using Dual-luciferase® Reporter Assay System (Promega) exactly following the manufacturers recommendations. Activity was measured on a TECAN Infinite M200Pro using an automated injector.

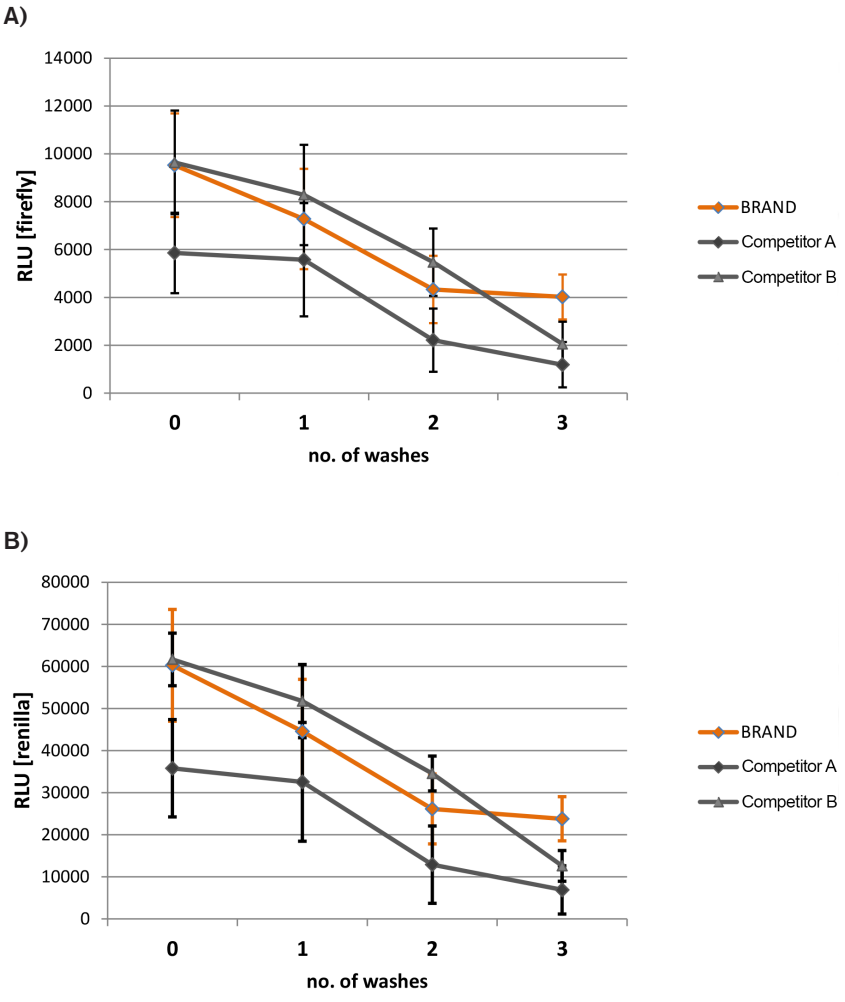


Figure: Cell loss after washing is directly proportional to the reduction of luminescence signal created by the activity of firefly (A) or *Renilla* (B) luciferase. Data are background corrected and show mean with standard deviation (Statistics: Grubbs outlier test (p=0.05; n=16).

Results

To ensure an equal pipetting strength during washing an electronic multichannel pipette was used. In this case the only variable is the TC culture surface of different manufacturers. The relative luminescence (RLU) generated by firefly and *renilla* luciferase shows that the cellGrade™ plus surface support proliferation of transfected cells to the same extent when compared to advanced TC-treated microplates from well known competitors. However, cell loss on cellGrade™ plus surface was minimized after 3 washing steps in comparison to competitors.

Conclusion

Solid white microplates with cellGrade™ plus surface keep transfected cells attached even under harsh washing conditions. Therefore this cell culture surface of the BRAND*plates*® is a perfect option to minimize cell loss during experiments with repetitive washing steps.

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