Application Note

High yields of transfected cells with BRAND*plates*® cellGrade™ premium surface

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Introduction

Transfection is defined as non-viral DNA/gene delivery into eukaryotic cells performed by several chemical, physical or biological methods. The subsequent exogenous expression of a tagged protein in cell culture is a well established approach to investigate function and localization of the protein of interest. In normal culture medium, nucleases present in serum could degrade DNA while other serum components tend to form complexes with nucleic acids, thereby reducing the availability of DNA for transfection [Ref.1]. To avoid such interference, serum free culture medium is required for successful transfections. However, serum deprivation can reduce cell viability, proliferation and attachment. To partially compensate for these negative effects arising from serum deprived culture conditions, special modifications of cell culture surfaces have been developed to support cell attachment and increase cellular yields after transfection.
Here we compare 3 different microplate surfaces regarding their ability to support proliferation and attachment of transfected cells during washing steps. It is shown, that on the cellGrade™ premium surface transfected cells were retained in same quantity when compared to 96-well microplates of other manufacturers.

Material & Methods

HEK293.EBNA cells were cultured in DMEM 4.5 g/L Glucose with L-glutamine supplemented with 10 % fetal bovine serum and 100 units/mL penicillin/streptomycin. Cells were seeded in comparable tissue culture treated black 96-well microplates with transparent bottom and grown at 37°C with 5% CO_2. A total of 200 ng/well GFP-encoding plasmid-DNA pEGFP-C1 was used to transfect cells using 40 kDa linear polyethylenimine at a ratio of 1:3 DNA:PEI40 24 hrs later [Ref.2]. After an incubation of 72 hrs culture medium was changed to PBS and one set of cultures from each microplate was washed additional 2 times with 200 µL PBS at 37 °C using an electronic multi-channel pipette at lowest dispensing speed in order to not disturb the cell monolayer. For read-out a TECAN Infinite® M200 PRO was used to detect the remaining relative fluorescent units (RFUs) at ex485/em535 nm. The detector of the plate reader was adjusted according to the highest signal intensity to be measured.

Results

A transfection mastermix was used to transfect cultured cells on different plates in order to achieve comparable transfection efficiency (Fig.1).

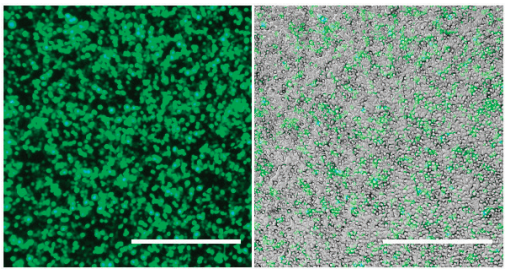

Figure 1. Example of transfected HEK293.EBNA cells expressing GFP 72 hrs post-transfection. Scale bar 500 µm.

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 quantification of relative GFP fluorescence units shows that cellGrade™ premium surface promote proliferation of transfected cells and retain GFP expressing cells after washing to the same extent as TC-treated microplates from competitors.

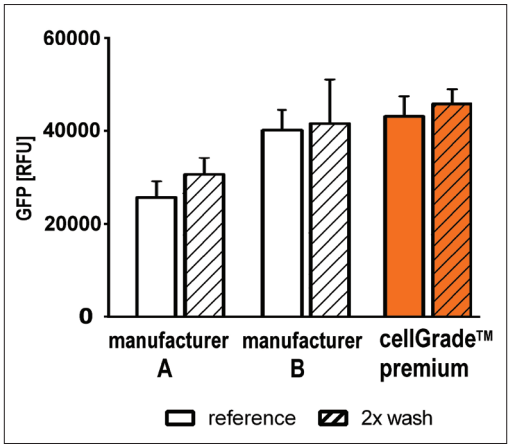

Figure 2: Measurement of GFP relative fluorescence units (RFU) shows the good performance of BRAND*plates*® cellGrade™ premium surface in promoting proliferation and attachment of transfected HEK293.EBNA cells.

Conclusion

BRAND*plates*® cellGrade™ premium surface improve experimental performance when cell proliferation or cell binding to culture surface is critical.

References:

1: D. Llères, J.M. Weibel, D. Heissler, G. Zuber, G. Duportail, Y. Mély, Dependence of the cellular internalization and transfection efficiency on the structure and physicochemical properties of cationic detergent/DNA/liposomes, J. Gene. Med. 6 (2004) 415–428.

2: SP. Huh et al., Optimization of 25 kDa linear polyethylenimine for efficient gene delivery, Biologicals. (2007), 35(3):165-71.

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