APPLICATION NOTE No. 326 I April 2014

Eppendorf 96-Well Cell Culture Plate – A simple method of minimizing the edge effect in cell-based assays

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Abstract

Cell-based assays in multi-well plates are a powerful tool which is widely used in drug discovery, tissue engineering and academic research. Consistency in the performance of these assays is crucial in order to obtain reliable data and to make valuable scientific statements. In this context evaporation of cell culture medium plays a critical role in multi-well based cellular assays because it can deteriorate assay performance. As evaporation mainly affects the peripheral wells of a plate this phenomenon is called the edge effect. With the Eppendorf 96-Well Cell Culture Plate evaporation from the wells can be reduced to a minimum. The plate offers two options for insulating the wells. A moat surrounding the edge wells and the complete chimney design of the inner wells allow filling of the complete inter-well space with liquid. Together this results in a more homogeneous distribution of temperature and humidity throughout the plate and reduces the edge effect.

Figure 1: The Eppendorf 96-Well Cell Culture Plate, filling options:
A: outer moat filled with liquid to insulate specifically the edge wells; B: insulation of all 96 wells by filling the complete inter-well space

Introduction

The analysis of cellular responses using cell-based assays is of increasing importance in almost all fields of biological science. The possibility to adapt cell-based assays to high throughput processes makes these assays indispensable e.g. in pharmaceutical research where multi-well based drug screenings in living cells are used to identify lead compounds. The significance and value of the obtained data relies on the consistency of the performed assays.

There are many sources of assay inconsistency in multi-well plates including variations in cell plating as well as differences in cell growth and adherence. Some of these aspects can be avoided by optimizing routine procedures in the laboratory. Possible measures include the implementation of adequate mixing and dissociation of the cells, the prevention of air bubble formation during cell seeding and the determination of optimal growth conditions concerning media formulation and seeding density. However, the edge effect can rarely be addressed by these precautions.

The causes of the edge effect are complex. Temperature differences across the plate and evaporation effects in the edge wells during incubation have been described as two possible influencing factors for irregular patterns of cell growth and distribution especially in the plate periphery [1; 2]. It is assumed that evaporation in the outer wells may lead to an accumulation of medium components (e.g. salts), thereby affecting cell metabolism. Varying cell responses to e.g. temperature gradients across the plate might occur when the plate is placed in the incubator resulting in a faster thermal equilibration of the outer wells. Both factors – evaporation and temperature gradient – may lead to assay inconsistencies and higher variances between individual assays, thus resulting in unreliable data.

To prevent the edge effect different methods have been described. One approach to minimize the impact of a temperature gradient is the pre-incubation of the plate with freshly seeded cells for 1–2 hours at room temperature which results in a more even cell distribution and adhesion pattern [3]. However, this procedure might be critical depending on the cell type used with regard to cell viability. For applications where long-term incubation is needed, products like humidity chambers and temperature chambers are available from different manufacturers. The purpose of these chambers is the creation of a constant micro-environment by reducing evaporation to a minimum and achieving an almost consistent humidity and temperature level.

Another very common method to circumvent the edge effect is to avoid seeding cells in the peripheral wells at the expense of sample throughput and efficiency. By discarding the outer wells of a 96-well plate the number of wells for analysis is reduced to 60 which results in a decrease of 38 % per plate. This results in higher investments for consumables, incubator space and time.

In the following, a simple and convenient method to minimize evaporation by utilizing the outer moat and the inter-well space of the Eppendorf 96-Well Cell Culture Plate is described. This application note compares the performance of Eppendorf 96-Well Cell Culture Plates with 96-well cell culture plates from different manufacturers concerning well-to-well variability in evaporation.

Material and Methods

a.) Preparation of the plates

For evaluating evaporation in 96-well plates the Eppendorf Cell Culture Plate as well as three 3 competitor plates were tested (n=2).

The outer moat of the Eppendorf plate was filled with 1 x 5 mL of PBS. The inner inter-well space was filled with 1 x 8 mL of PBS. The plate from competitor A provides a moat with four reservoirs that can be filled with liquid. 1.75 mL of PBS was filled into each of the four reservoirs (7 mL in total).

The plates were equilibrated in an Eppendorf New Brunswick™ Galaxy® CO_2 incubator at 37 °C and 5 % CO_2 in humidified atmosphere.

The plates of competitor B and C do not offer the option of insulating the wells by filling the inter-well space and/or an outer moat, therefore no preparation steps have been performed with these plates.

b.) Measurement of evaporation

After equilibration each plate was filled with 200 µL sterile water per well using the automated pipetting system ep*Motion*® 5075m. The plates were incubated under standard cell culture conditions (37 °C, 5 % CO_2, humidified atmosphere).

Following 5 days of incubation, plates were removed from the incubator and equilibrated at room temperature for 30 min. 0.01 % crystal violet solution was added (20 µL/well) and mixed by resuspension. 100 µL of this mixture was transferred to each well of an Eppendorf Microplate (VIS 96/F). For generating calibration curves crystal violet standards were prepared. Absorbance at 600 nm was measured using the Eppendorf PlateReader AF2200.

The percentage of evaporation in each well after 5 days under standard cell culture conditions was calculated by referring to the respective calibration curve. Mean values of two replicates are depicted in figure 2.

Results and Discussion

During longer incubation periods evaporation in 96-well plates is a critical factor. Especially in the edge wells liquid loss becomes apparent, because these wells are not completely surrounded by neighboring wells. Insulating the edge wells with liquid can reduce evaporation, as shown in table 1. Without insulation the edge wells of the Eppendorf 96-Well Cell Culture Plates show an average evaporation of only 1.8 % after 5 days of incubation under standard cell culture conditions. The low evaporation independent of the moat filling is probably a result of the optimized fit between lid and plate. Filling the surrounding moat of the Eppendorf plate® with liquid further reduces evaporation to less than 1 %. As shown in figure 1 the complete chimney-well design of the Eppendorf plate allows additional filling of the inter-well space minimizing evaporation in the edge wells to only 0.3 %. In addition the insulation of each single well leads to slower cooling of the media in the wells when the plate is handled outside the incubator (e.g. during microscopy).
Any liquid e.g. sterile PBS, water or cell culture medium is suitable for filling the moat and the inter-well space. The design of the Eppendorf Cell Culture Plate makes the filling easy and convenient in just one pipetting step.

Filling option	Evaporation in edge wells [%]
no filling	1.8
surrounding moat (5 mL)	0.9
surrounding moat (5 mL) and inter-well space (8 mL)	0.3

Table 1: Effective insulation reduces evaporation in Eppendorf 96-Well Cell Culture Plates. Average evaporation in edge wells after 5 days of incubation under standard cell culture conditions.

A comparison of different 96-well cell culture plates (see figure 2 and table 2) shows that the Eppendorf Cell Culture Plate is the only plate that effectively minimized evaporation. Competitor A offers an option of partly insulating the peripheral wells (see Material and Methods), but still showed an edge effect although more moderate compared to competitors B and C. In all competitor plates a very high percentage of evaporation was observed especially in the four corner wells.

96-well plate	Filling option	Evaporation in edge wells [%]
Eppendorf	yes	0.3
Competitor A	yes	3.0
Competitor B	no	8.3
Competitor C	no	4.8

Table 2: Average evaporation in edge wells of 96-well plates.

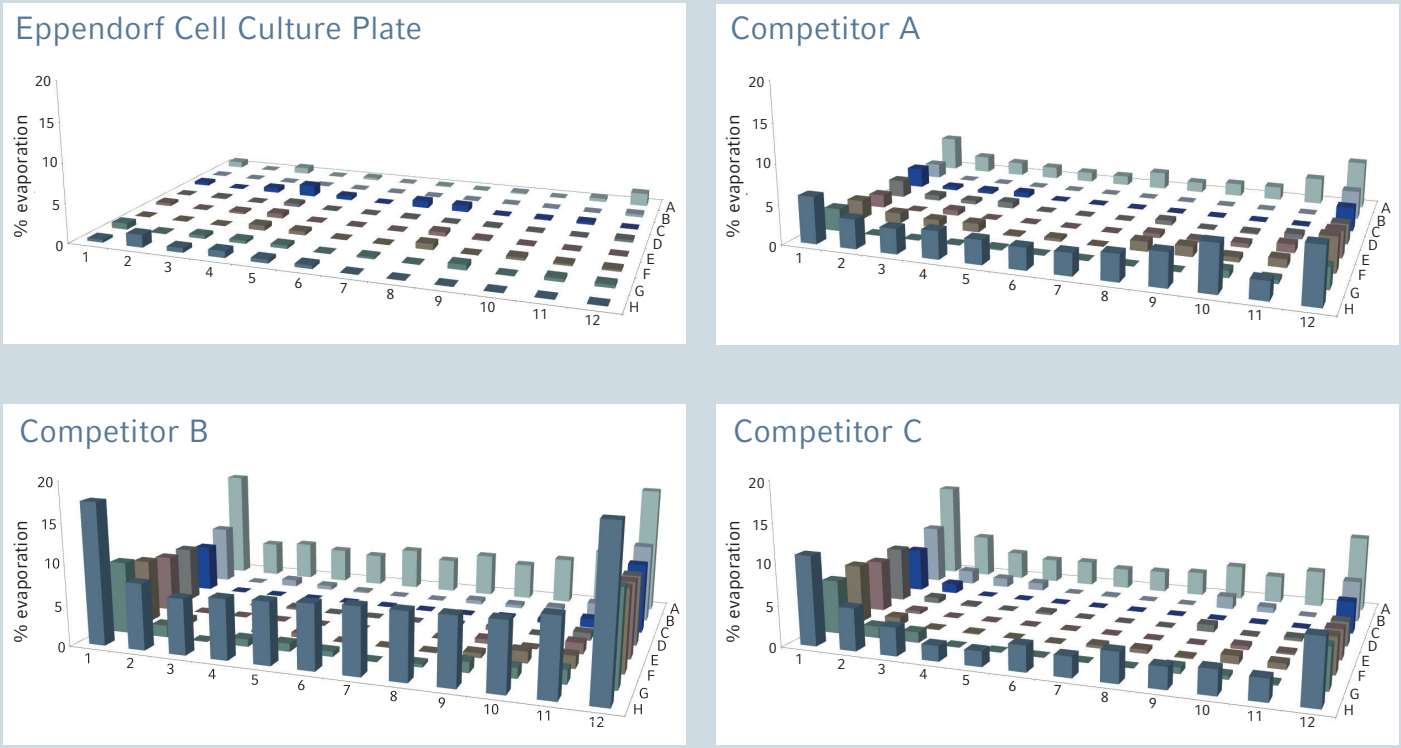

Figure 2: Comparison of evaporation in different 96-well plates.
Liquid loss inside each well was determined after 5 days of incubation under standard cell culture conditions.

Filling the complete inter-well space of the Eppendorf Cell Culture Plate with liquid contributes to a more homogenous humidity and temperature stability within the plate. This leads to a decrease in evaporation and condensation and therefore reduces the edge effect.

Here we present a simple and convenient method to minimize evaporation and therefore reducing the edge effect in plate-based assays utilizing 96-well plates. Only the design of the Eppendorf Cell Culture Plate offers two different options of insulating the edge wells and the central wells (see figure 1), leading to a homogeneous distribution of temperature and humidity.

Therefore the Eppendorf Cell Culture Plate is best suited for every cell-based assay as liquid loss due to evaporation across the plate can be effectively minimized and the whole plate can be used without fearing the impact of the edge effect.

Literature

[1] A simple and cost efficient method to avoid unequal evaporation in cellular screening assays, which restores cellular metabolic activity. Walzl A, Kramer N, Mazza G, Rosner M, Falkenhagen D, Hengstschläger M, Schwanzer-Pfeiffer D, Dolznig H. Int J Appl Sci Technol. 2012 June; 2(6)

[2] A pitfall of the 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethonyphenol)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay due to evaporation in wells on the edge of a 96 well plate. Patel MI, Tuckerman R, Dong Q Biotechnology Letters 2005; 27: 805–808

[3] A simple technique for reducing edge effect in cell-based assays. Lundholt BK, Scudder KM, Pagliaro L Journal of Biomolecular Screening 2003; 8: 566

Ordering information

Description	Order no. international	Order no. North America
Eppendorf Cell Culture Plate, 96-Well TC treated (with lid, flat bottom, sterile, free of detectable pyrogens, RNase & DNase, DNA. Non-cytotoxic) individually wrapped, 80 plates per case	0030 730.119	0030730119
Eppendorf Cell Culture Plate, 96-Well non-treated (with lid, flat bottom, sterile, free of detectable pyrogens, RNase & DNase, DNA. Non-cytotoxic) individually wrapped, 80 plates per case	0030 730.011	0030730011
Eppendorf Microplate VIS 96/F (wells clear, border colorless), PCR clean, 40 plates (4 bags x 10 plates)	0030 730.020	0030730020
New Brunswick™ Galaxy® 170R CO_2 incubator (170 Liter, with high temperature disinfection) – 230 V/60 Hz – 120 V/60 Hz	 CO170R-230-1000 CO170R-120-1000	 CO170R-120-1000
New Brunswick™ Galaxy® 48R CO_2 incubator (48 Liter, with high temperature disinfection) – 230 V/60 Hz – 120 V/60 Hz	 CO48R-230-1000 CO48R-120-1000	 CO48R-120-1000
PlateReader AF2200 (Dual-mode plate reader for UV/VIS absorption and fluorescence intensity) – 230 V/50–60 Hz – 120 V/ 50–60 Hz	 6141 000.002 6141 000.010	 6141000010
ep*Motion*® 5075m (Automated pipetting system, basic device incl. MagSep module, ThermoMixer®, epBlue™ software and Prep assistant, mouse, waste box, 1 µL–1 mL, 100–240 V/50–60 Hz)	5075 000.305	5075006024

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