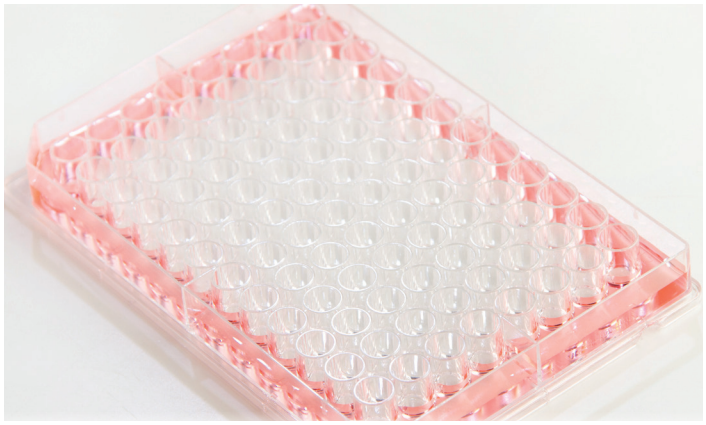
Preventing edge effect during extended culture in microplates with a unique perimeter moat design

Abstract

Preventing edge effect during prolonged microplate culture is a common concern of researchers. When considering the small volume of medium used in each well, minimizing evaporation is both critical and challenging, especially in the outer wells that are most susceptible. Here we present the Thermo Scientific™ Nunc™ Edge 2.0 96-Well Plate, with a perimeter moat design, as an effective and efficient solution for significantly reducing evaporation during extended microplate culture. In this application study, evaporation levels are compared between conventional 96-well plates (plates without an evaporation moat) and the Nunc Edge 2.0 plate. Additionally, cell viability is assessed and compared between 96-well plates with and without an evaporation reservoir, to further demonstrate the positive impact that Edge 2.0 plates have on consistency and productivity of cell-based assays.

Introduction

Edge effect is caused when evaporation occurs in the perimeter wells of a microplate during prolonged culture. It changes pH, osmolality, and concentration of the medium and its constituents, which all affect cell viability and function. There are, currently, few



options to minimize evaporation during extended cell culture, and for this reason it is common practice to leave the 36 perimeter wells of a 96-well plate empty. Unfortunately, this workaround reduces the capacity of a standard plate by 37.5%. The uniquely designed Edge 2.0 96-Well Plate overcomes this issue with a perimeter moat in which sterile fluid can be added. When filled, this moat significantly reduces evaporation across the entire plate, most notably in the outer wells, resulting in improved viability of all cells cultured in the plate.

Materials and methods

Material*	Brand	Cat. No.
Nunc Edge 2.0 96-Well Plate	Thermo Scientific	167425
Dulbecco's Modified Eagle Medium (DMEM)	Gibco	10938025
L-Glutamine, 200 mM	Gibco	25030024
Penicillin-Streptomycin (10,000 U/mL)	Gibco	15140122
Fetal Bovine Serum (FBS)	Gibco	10099141
Trypsin-EDTA (0.25%)	Gibco	25200072
alamarBlue Cell Viability Reagent	Invitrogen	DAL1100
A549 Human Carcinoma Cell Line	ATCC	CCL-185
Methyl Violet	Ampliqon	AMPQ00314.1000

* Other 96-well flat-bottom cell culture plates of brands N, C, and E were used for the comparison studies.

Evaporation study

To study evaporation, 1.7 mL of sterile water was added to the Edge 2.0 plate's evaporation moat prior to incubation. The Edge 2.0 and standard 96-well microplates were filled with 100 µL/well of a 0.1% methyl violet solution. All plates were kept in a CO_2 (5%) incubator for 4 days at 37°C with a humidified atmosphere. During the incubation period the incubator door was opened for 15 seconds every hour, 7 times a day, to simulate common conditions of use. Whole-plate evaporation was determined by weighing plates before and after incubation. Evaporation from individual wells was determined colorimetrically by transferring 50 µL of the methyl violet solutions from each well to standard 96-well plates before reading optical density at 590 nm.

Cell viability assay

A549 human carcinoma cells (9,000 cells/mL, 100 µL/well) were incubated in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, and 2 mM L-glutamine. The evaporation reservoirs in both the Edge 2.0 and brand E plates were filled with sterile water. After a 4-day incubation at 37°C in a humidified, 5% CO_2 incubator, A549 cell viability was determined by an Invitrogen™ alamarBlue™ assay. The variation in cell viability for each well was calculated as the percent variance from the plate mean.

Results and discussion

When filled with sterile water, the perimeter moat of the Nunc Edge 2.0 plate prevents pronounced evaporation, as demonstrated by significant reductions in whole-plate evaporation during the 4-day incubation period—surpassing the performance of conventional 96-well plates (Figure 1). When individual wells were evaluated, evaporation in the most susceptible 36 perimeter wells was markedly reduced in the Edge 2.0 plate compared to evaporation in perimeter wells of the conventional brand N and brand C 96-well plates. The differences between the

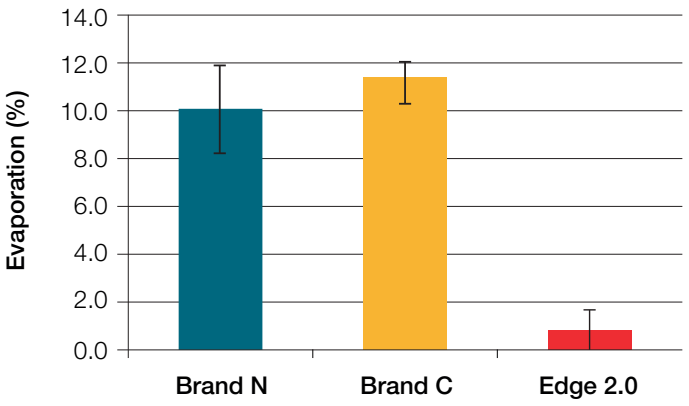


Figure 1. Whole-plate evaporation. The built-in perimeter moat of the Nunc Edge 2.0 plate significantly reduces evaporation over the entire surface of the plate, compared to conventional 96-well plates from brand N and brand C.

perimeter wells and the center wells in the Nunc Edge 2.0 plate are minimal, indicating the elimination of edge effect (Figure 2). Further studies show that evaporation-induced edge effect in conventional 96-well plates negatively impacts viability of A549 cells, leading to high well-to-well variance. The Nunc Edge 2.0 plate with a larger built-in perimeter moat more effectively improves well-to-well variance, compared to conventional 96-well plates (Figure 3).

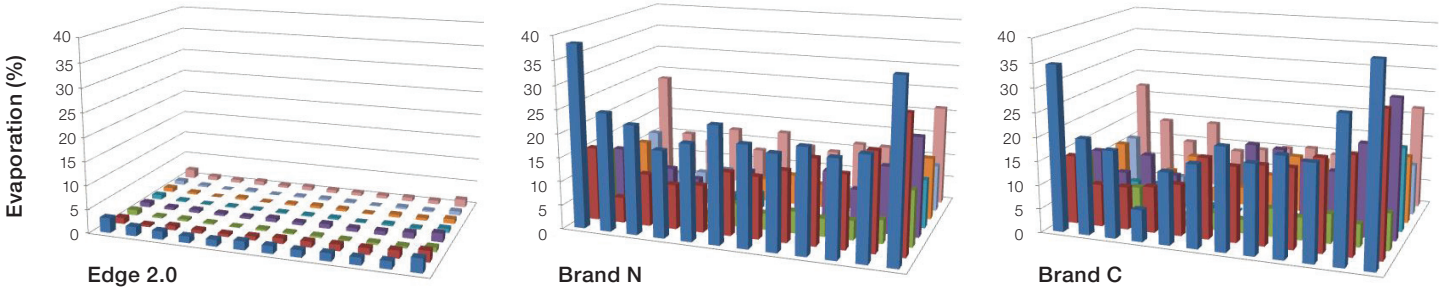


Figure 2. Individual-well evaporation. The built-in perimeter moat of the Nunc Edge 2.0 plate effectively eliminates the edge effect caused by evaporation, which is seen in the conventional plates of brands N and C.

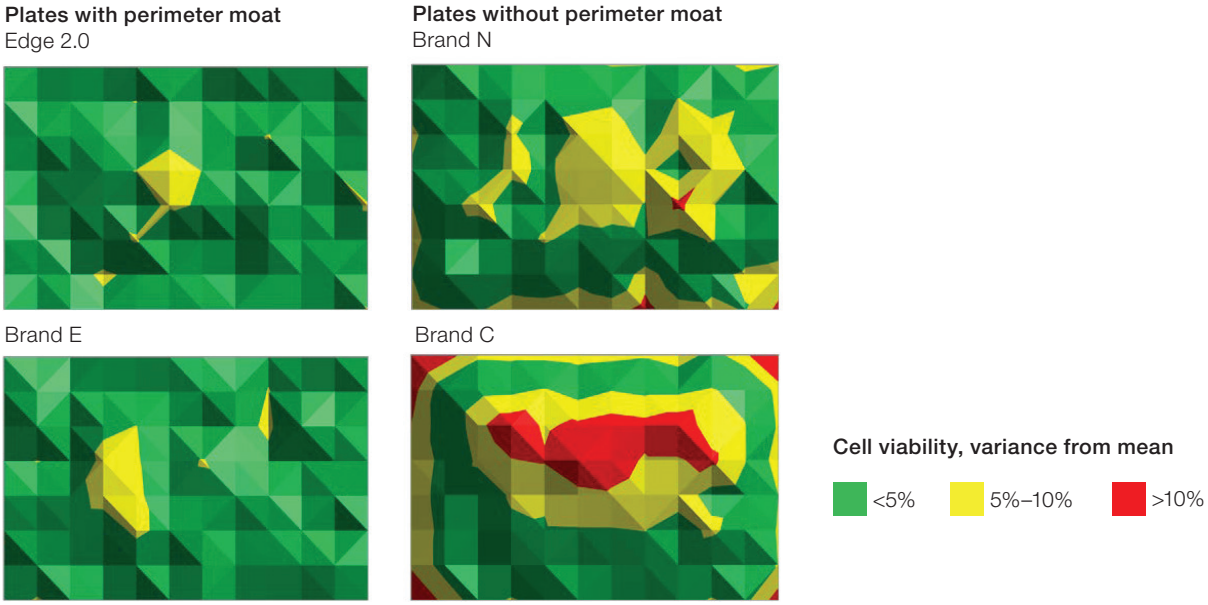


Figure 3. Cell viability. Variance in cell viability is minimized in plates with a built-in perimeter moat, compared to those with no moat. The Nunc Edge 2.0 plate with the larger built-in moat more effectively improves cell viability and consistency across the entire plate.

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Conclusions

- The Nunc Edge 2.0 plate with its built-in perimeter moat significantly reduces evaporation and the resultant edge effect, during prolonged incubation.
- The moat of the Nunc Edge 2.0 plate enables the use of all 96 wells, enabling researchers to potentially reduce costs and plastic waste compared to conventional plates.
- The large moat of the Nunc Edge 2.0 plate provides a highly effective evaporation barrier, improving well-to-well consistency.

Ordering information

Surface	Color	Well volume (µL)	Sterile	With lid	Units per pack/case	Cat. No.
Nunc Edge 2.0 96-Well Plates						
Nunclon Delta treated*	Clear	400	Yes	Yes	1/50	167425
			Yes	Yes	10/160	167542
			Yes	No	1/50	167574
			Yes	No	10/160	167554
Nontreated	Clear	400	Yes	Yes	1/50	267427
			Yes	Yes	10/160	267544
			Yes	No	1/50	267576
			Yes	No	10/160	267556
			No	No	10/160	267566
			No	Yes	10/160	267578

* Thermo Scientific™ Nunclon™ Delta is a Nunc certified cell culture–treated surface that facilitates cell attachment and growth—perfect for most applications with adherent cell cultures.

Find out more at **thermofisher.com/edgeplate**

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