

Minimizing evaporation in microplate with the advanced design of a large built-in moat

Key Words

Thermo Scientific Nunc Edge 2.0 96-well plate, edge effect, cell-based assay, built-in moat, microplate

Abstract

A common complaint around microplate cell culture is the problem with evaporation. Due to the small volume of medium used within each well, those located around the perimeter of the microplate are especially susceptible. Here we present the Thermo Scientific™ Nunc™ Edge 2.0 plate with a built-in moat surrounding the perimeter wells as an ideal solution to prevent evaporation. In this application study, the evaporation level is compared between the conventional 96-well plates and the Nunc Edge 2.0 plate. Furthermore, cell viability is assessed in plates with and without the moat to demonstrate the positive effect of the evaporation barrier on cell-based assays.

Introduction

The “edge effect” in the perimeter wells of a microplate is caused by evaporation during prolonged culture. It changes media pH, osmolality and the concentration of the media constituents, therefore affecting cell viability and function. Few options exist to minimize evaporation during extended cell culture. A common practice is to leave the 36 perimeter wells in a 96-well plate unused, sacrificing 37.5% of the plate capacity. The edge design



in the Thermo Scientific Nunc Edge 2.0 96-well plate overcomes this issue by incorporating a built-in moat to which sterile fluid can be added. This feature significantly reduces evaporation in the entire plate especially in the perimeter wells, directly improving the wellbeing of the cells cultured in the plate.

Experimental Details

Materials *	Brand	Catalog #
Thermo Scientific Nunc Edge 2.0 plates	Thermo Scientific	167425
Dulbecco's Modified Eagle Medium (DMEM)	Gibco	10938-025
L-Glutamine, 200 mM	Gibco	25030-024
Penicillin-Streptomycin (10,000 U/mL)	Gibco	15140-122
Fetal Bovine Serum (FBS)	Gibco	10099-141
Trypsin-EDTA (0.25%)	Gibco	25200-072
AlamarBlue® Cell Viability Reagent	Invitrogen	DAL1100
Human Carcinoma Cell line, A549	ATCC	CCL-185
Methylviolet	Amplicon	AMPQ00314.1000

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

Evaporation study

To study evaporation, 1.7 mL sterile water was added to the built-in moat in the Nunc Edge 2.0 plate before incubation. All 96-well microplates were filled with 100 μ L/well methylviolet solution (0.1%). The plates were incubated for 4 days at 37°C in a humidified atmosphere of 5% CO₂ incubator. During incubation, the incubator door was opened for 15 seconds every hour 7 times a day to simulate common use conditions. Whole plate evaporation was determined by appropriate plate weighing before and after incubation. Evaporation from individual wells was determined colorimetrically by transferring 50 μ L/well methylviolet aliquots to standard 96-well plates before OD reading at 590 nm.

Cell viability assay

Human carcinoma A549 cells (9000 cells/mL, 100 μ L/well) were incubated in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, and 2 mM L-glutamine. The moats in the Nunc Edge 2.0 plate and similar plate of Brand E were filled with sterile water. After 4-day incubation at 37°C in a humidified 5% CO₂ incubator, A549 cell viability was determined by AlamarBlue assay. Variation of cell viability in each well was calculated as percent variance against the plate mean.

Results and Discussion

The water-filled moat surrounding the Nunc Edge 2.0 plate prevents pronounced evaporation as demonstrated by the significant reduction in whole plate evaporation during the 4-day incubation, surpassing the performance of conventional 96-well plates (Figure 1). When evaluated for each individual wells in the plate, evaporations in the most susceptible 36 perimeter wells are markedly reduced in the Nunc Edge 2.0 plate comparing to the perimeter wells in the conventional plates of Brand N and Brand C. The differences between the perimeter wells and the center wells in the Nunc Edge 2.0 plate are minimal, eliminating the “edge effect” in the microplate (Figure 2). Further studies demonstrate that evaporation-induced “edge effect” in the conventional plates negatively impacts A549 cell viability and leads to high variance from well-to-well. The Nunc Edge 2.0 plate with the large built-in moat not only adequately minimizes the well-to-well variance seen in the conventional plates, but also outperforms Brand E plate with a narrow built-in moat in cell viability consistency across the entire plate (Figure 3).

Whole Plate Evaporation

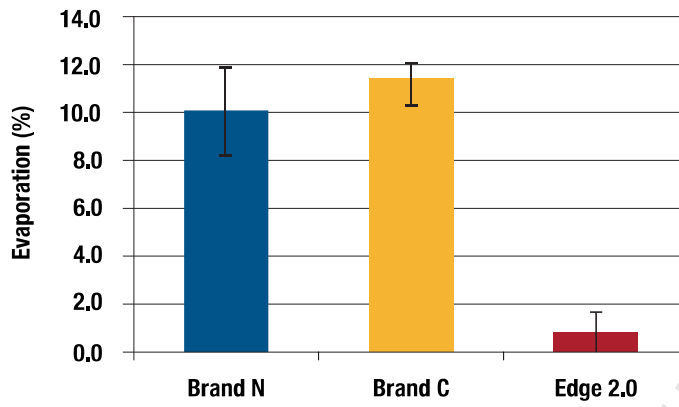


Figure 1. The built-in moat in the Nunc Edge 2.0 plate significantly reduces whole plate evaporation comparing to the conventional 96-well plates of brand N and brand C.

Individual Well Evaporation

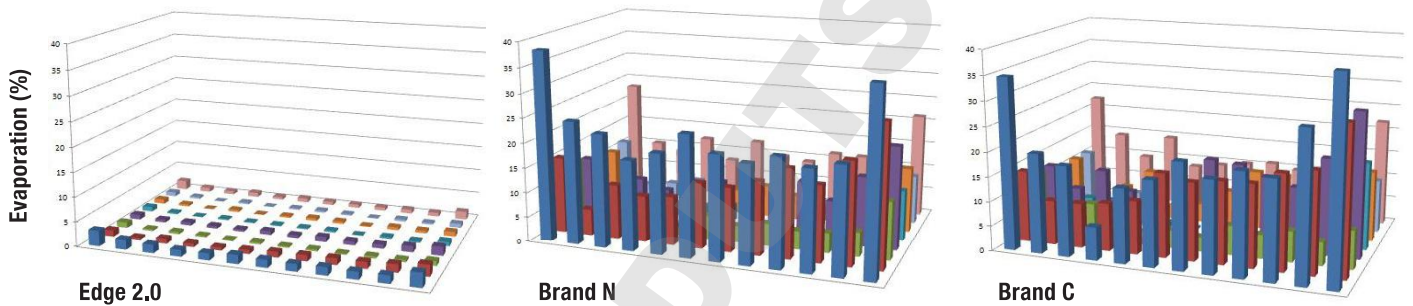


Figure 2. The built-in moat in the Nunc Edge 2.0 plate effectively eliminates the “edge effect” caused by evaporation as seen in the conventional plates of Brand N and Brand C.

Cell Viability

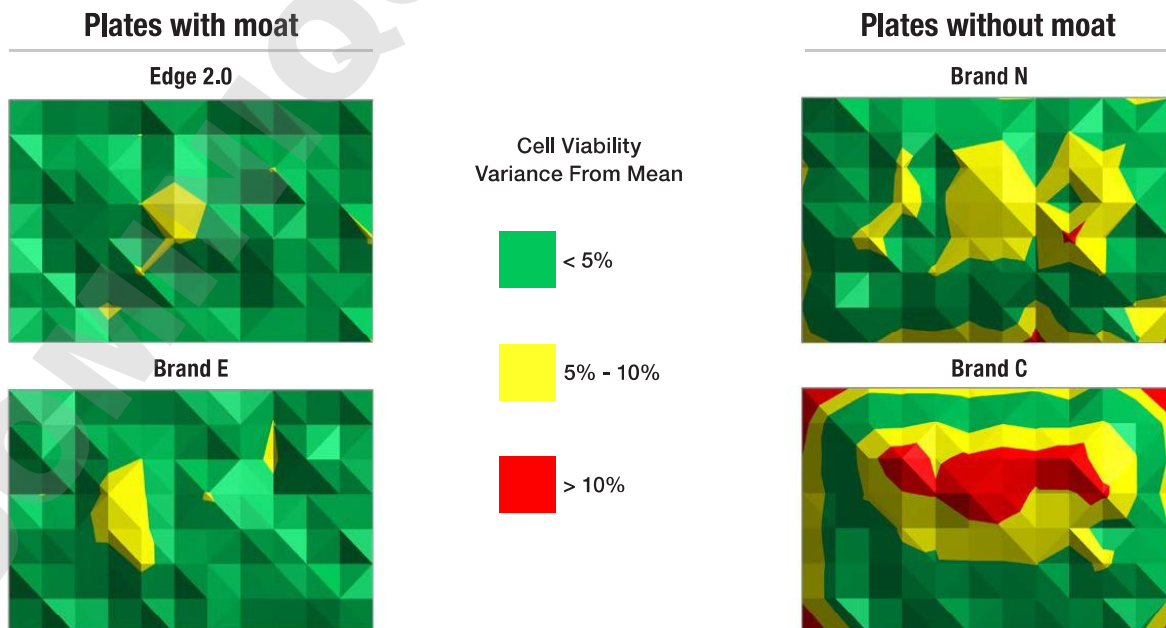


Figure 3. Cell viability variance is minimized in plates with the built-in moat comparing to those without the moat (Brand N and Brand C). The Nunc Edge 2.0 plate outperforms Brand E plate with narrow moat in cell viability consistency across the entire plate.

Conclusion

- Thermo Scientific Nunc Edge 2.0 plate with the built-in moat significantly reduces evaporation and the resultant “edge effect” during prolonged incubation.
- The surrounding moat in the Nunc Edge 2.0 enables the use of all 96 wells in the microplate without the concern of evaporation, leading to improved productivity for cell-based assays.
- The large built-in moat in the Nunc Edge 2.0 plate provides the most effective evaporation barrier among plates with moat designs, demonstrating the highest performance in well-to-well consistency.

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Ordering information

Edge 2.0 96-Well Plates

Cat. No.	Surface	Color	Total vol. µl/well	Sterile	With lid	Units/Case/Pack
167425	Nunclon Delta Treated	Clear	400	Yes	Yes	1/50
167542	Nunclon Delta Treated	Clear	400	Yes	Yes	10/160
167574	Nunclon Delta Treated	Clear	400	Yes	No	1/50
167554	Nunclon Delta Treated	Clear	400	Yes	No	10/160
267427	Non treated	Clear	400	Yes	Yes	1/50
267544	Non treated	Clear	400	Yes	Yes	10/160
267576	Non treated	Clear	400	Yes	No	1/50
267556	Non treated	Clear	400	Yes	No	10/160
267566	Non treated	Clear	400	No	No	10/160
267578	Non treated	Clear	400	No	Yes	10/160

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.

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