Application Note

Thermo Scientific Nunc Edge Plate Technical Information

Intrawell Cell Distribution in MicroWell Edge Plates

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It is a well known fact that temperature gradients and vibrations during cell settlement in cell culture flasks and plates may cause uneven cell distribution patterns on the growth surfaces [1, 2]. Therefore, all ingredients assembled should be left in absolute tranquility (i.e. no temperature gradients, no vibrations, and no ventilation) during cell settlement. As communicated elsewhere [3], this is most easily accomplished by pre-incubation of the seeded culture at room temperature (RT).

The significance of the evaporation reservoir in the Thermo Scientific Nunc Edge Plate in relation to the uneven cell distribution was investigated with either 200 or 100 µL MDCK cell suspension per well according to the following 4-plate test set-up distinguishing four different situations framed in red:

Plate Number		1	2	3	4
Conditions	Plate	RT	RT	RT	RT
	Cell Suspension	RT	RT	RT	RT
	Reservoir	RT water*	RT water*	Empty	Empty
	Pre-incubation	none	2 hrs at RT	none	2 hrs at RT
	Incubation**	37°C	37°C	37°C	37°C

* 1.75 mL per reservoir compartment
**5% CO_2 in air

Figure 1 shows the results with 200 µL cell suspension per well, where it is seen that without pre-incubation (Plates 1 and 3) outward "half-moon" cell accumulations occur in the edge wells, but to a lesser degree in the plate with the reservoir filled (Plate 1) compared to the plate with an empty reservoir (Plate 3). In the latter case, with an empty reservoir and no pre-incubation, additional patterns occur in the edge wells, which may stem from incubator vibrations. However, both phenomena are eliminated by pre-incubation (Plates 2 and 4). Therefore, the reservoir content may to some extent act as a "buffer" against uneven cell distribution in the (edge) wells but has no significance if pre-incubation is employed.

Figure 2 shows the results with 100 µL cell suspension per well, where edge effects are largely absent, thus indicating a volume-dependent reverse effect. This may be explained by the shorter settling distance and time, making the cell distribution less sensitive to thermal disturbances in the wells.

Figure 3 theoretically explains the cell distribution skewing, observed with 200 µL cell suspension, by temperature gradients upon incubation at 37°C. In model experiments with suspended, descending particles in water it has been demonstrated that a convection stream circulating as illustrated would indeed occur when heating the side of the vessel, and it would "sweep" the particles into the heated corner of the vessel.

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Plate 1 - Reservoir filled, no pre-incubation

Plate 2 - Reservoir filled, pre-incubation

Plate 3 - Reservoir empty, no pre-incubation

Plate 4 - Reservoir empty, pre-incubation

Fig. 1. Edge Plates seeded with 200 µL MDCK suspension per well: Stained with crystal violet after incubation at 37°C for 3 days. See text for explanation.

Plate 1 - Reservoir filled, no pre-incubation

Plate 2 - Reservoir filled, pre-incubation

Plate 3 - Reservoir empty, no pre-incubation

Plate 4 - Reservoir empty, pre-incubation

Fig. 2. Edge Plates seeded with 100 µL MDCK suspension per well: Stained with crystal violet after incubation at 37°C for 3 days. See text for explanation.

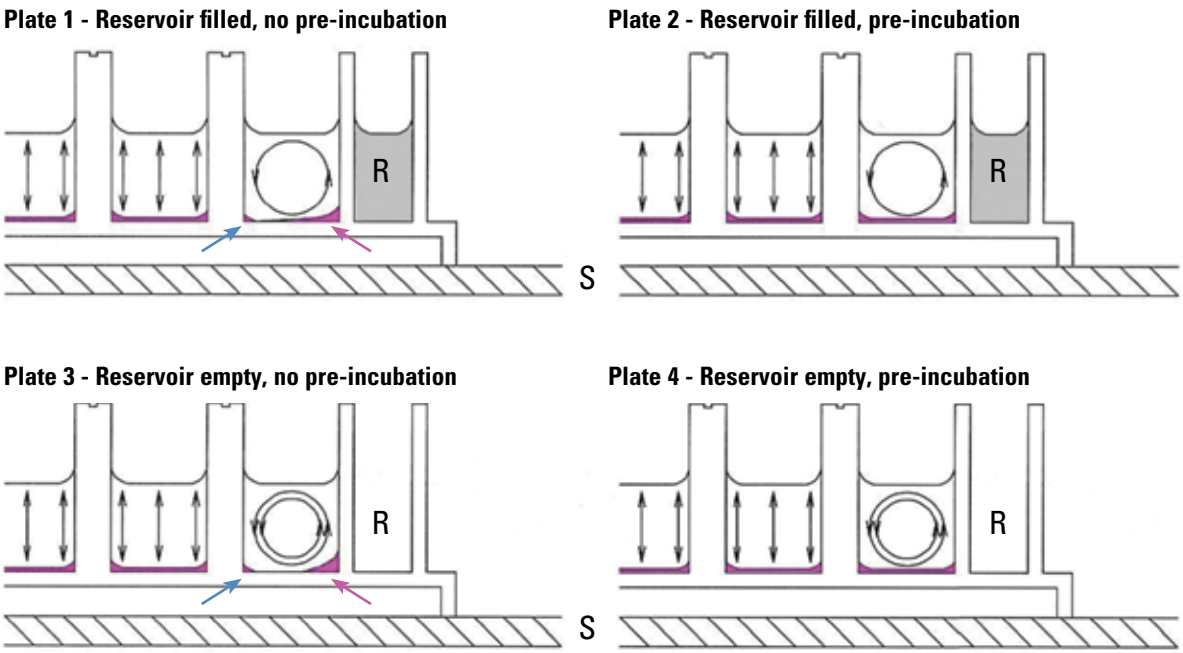

Fig. 3. Theoretical profile scenarios in Nunc™ Edge Plates with the reservoir (R) filled (top) or with an empty reservoir (bottom), when put on shelves (S) in 37°C incubator immediately after addition of cell suspension at RT (left), or after pre-incubation with cell suspension at RT (right). The uneven heating at the plate edges will create circulating convection streams in the edge wells (as opposed to the central wells having more homogeneous convection). Without pre-incubation, descending cells accumulate at the outward parts of the well corners (pink arrows) to a larger degree with empty reservoir than with the "buffering" effect of filled reservoir. With pre-incubation, where the cells have been allowed to settle in the absence of temperature gradients, convection streams will not skew the cell distribution pattern. A denser rim of cells all around the well corners (blue arrows) will always be observed reflecting the larger liquid column heights (thus more cells) at the well periphery than at the center.

Conclusion

In conclusion, the recommendation of tranquil pre-incubation during cell settlement is maintained for the Nunc Edge Plates on the condition that the reservoir content has the same temperature as the other ingredients during the pre-incubation. However, the extent of thermal disturbance is also dependent on cell type, thus with MRC5 cells we observed almost no such effect (results not shown).

With suspension volumes reduced to 100 µL per well, pre-incubation may be unnecessary, but users may normally avoid smaller volumes because evaporation would be more critical. This condition could be eliminated by using Edge Plates with the reservoir filled.

References:

1. Nielsen V. and Esser P. *Incubator Shelf "Images" in Monolayer Culture*. Nunc Bulletin No. 3, 2nd Ed. 1997. Visit: www.thermoscientific.com/edgeplate for more information.

2. Nielsen V. *Vibration Patterns in Tissue Culture Vessels*. Nunc Bulletin No. 2, 2nd Ed. 1997. Visit: www.thermoscientific.com/edgeplate for more information.

3. Lundholt B. K. et al. *A Simple Technique for Reducing Edge Effect in Cell-Based Assays*. Journal of Biomolecular Screening 8(5), 2003.

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