Technical Bulletin: 15

Substrate Performance Study of 96 and 384 Well Thermo Scientific Nunc Polymer Optical Bottom Plates

Abstract

A study was conducted to determine the performance of Thermo Scientific Nunc 96 and 384 well microplates. Plates were evaluated for auto-fluorescence background, cross-talk between wells, halo effect, optical clarity, and cell-binding capacity. Microplates made of a clear polymer film attached to an upper structure are usually treated post-manufacturing to increase surface reactivity in cell-based and protein binding assays. For automated high throughput screening (HTS) systems, high quality optical bottom plates assure accuracy and consistency, generating high signal-to-noise ratios.

Nunc™ 96 and 384 well black microplates outperformed other brands and are recommended for use in drug discovery, screening, genomics, and proteomics studies.

Introduction

96 and 384 well microplates have been widely used in clinical, diagnostic, pharmaceutical and biotechnology areas due to their capacity and compatibility with robotic automation systems. Although the Society for Biomolecular Screening (SBS) Standards Committee has established specifications for microplates to promote the consistency of the physical format, there are still other criteria critical in choosing the proper microplates for specific applications. Among these criteria are the optical quality of the polymer film, the binding capacity of substrate polymer and the ultimate method of detection in any specific assay system.

Among the different types of multi-well plates, 96 and 384 well polymer optical bottom plates are designed to provide better optical clarity in microscopic applications and in fluorometric/colorimetric assays. Most surfaces are treated to enhance cell adhesion or protein binding in such applications as cell growth/proliferation studies [1,2,3], cell adhesion studies [4,5,6] and cytotoxicity assays [7,8]. Surface treatment is a requirement for most assays used in the general areas of immunoassay [9], proteomics, genomics [10] and drug discovery screening [11]. In addition, substrate polymers are required to provide low absorbance (high transmission) in monochromic or spectroscopic detection and to express low auto-fluorescence background in the range of the most commonly used fluorescence tags (i.e. Texas red, Fluorescein SYBR Gold, SYBR Green). In some plates a halo effect can be seen in the form of auto-fluorescence at the edges of the well. The cause is overheating and pressure necessary in the application of the film to the base of the plate. By careful control of these parameters, the halo effect has been eliminated. The 96 and 384 well polymer optical bottom plates demonstrate excellent performance when compared to other polymer substrates.

Material and Methods

Samples of 96 well black microplates from an industry-wide cross-section of plate manufacturers were obtained commercially and labeled as Thermo Scientific Nunc, Brand A, Brand B, Brand C and Brand D. Samples of 384 well black microplates are Thermo Scientific Nunc and Brand E.

For auto-fluorescence background comparisons, images were captured using an Alpha Innotech instrument. 96 well microplates were inverted with the camera set up as 2.8F/0.8NA/ Excitation 365 nm/Emission filter 4-Fluorescein, SYBR Gold 520±40 nm, image capture/Normal~High, Contrast/10023 (white) and 0.5 (Gamma) and analyzed using FluorChem v2.0. 384 well microplates were viewed with the camera set up as 2.8F/0.8NA/ Excitation 365 nm/Emission filter 4-Fluorescein, SYBR Gold 520±40 nm, image capture/Normal~High, followed by brightness adjustment to 50%(+) using MGI Photo Suite II. For fluorescence intensity, the system was calibrated with a fluorescence standard kit (Hitachi), and a 96 well template was employed to obtain readouts.

Transmittance data was obtained using a standard spectrophotometer. BHK-21, a fibroblastic-like cell line derived from baby hamster kidney (ATCC CRL-8544), was grown in BME containing L-glutamine, Pen/Strep, 10% Tryptose phosphate broth and 10% bovine calf serum (BCS). V79, a fibroblastic-like cell line derived from Chinese hamster lung (ATCC CCL-93), was grown in MEM with L-glutamine, Pen/Strep, non-essential amino acids (NEAA) and

Thermo SCIENTIFIC

10% BCS. L929, a fibroblastic-like cell line derived from adult mouse lung (ATCC CCL-1), was grown in MEM with L-glutamine, Pen/Strep, NEAA and 10% BCS. HEL299, a diploid fibroblastic-like cell line derived from embryonic human lung (ATCC CCL-137), was grown in MEM with L-glutamine, Pen/Strep, sodium pyruvate, NEAA and 10% BCS. Hep-2, an epithelial cell line derived from human carcinoma (ATCC CCL-23) was grown in MEM with L-glutamine, Pen/Strep and 10% fetal bovine serum. Cells were seeded at a density of $2x10^4$ per well. After 20 hours of incubation, cells were fixed and stained using crystal violet.

Results

The optical properties of polymer optical bottom plates were compared with regard to fluorescence background, fluorescence readout, light transmittance and the halo effect. As shown in Figs. 1 and 2, the lowest auto-fluorescence background was achieved using Thermo Scientific Nunc plates (fluorescent intensity was calibrated using an external standard). In addition, excellent transmittance from 300-700 nm (>80%) was observed for Thermo Scientific Nunc plates (Fig. 3).

Fluorescent images of Thermo Scientific Nunc plates demonstrate both low auto-fluorescence and an absence of any observable halo effect (Fig. 4).

A variety of cell lines including BHK, V79, L929, HEL299 and Hep-2 grow with characteristic morphology on Thermo Scientific Nunc polymer optical bottom plates (Fig. 5). Both epithelial (Hep-2) and fibroblastic (L929) attach homogeneously to the Thermo Scientific Nunc polymer substrate.

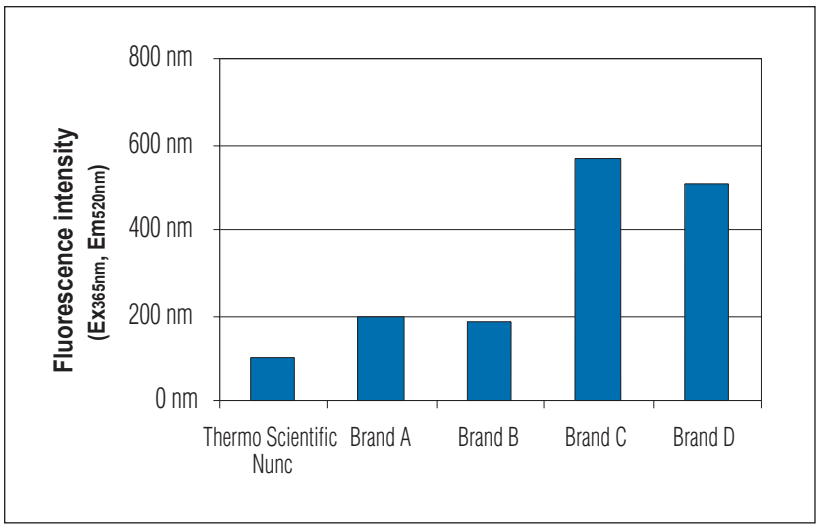

Fig. 1
The auto-fluorescence intensity of 96 well black microplates: Thermo Scientific Nunc, Brand A, Brand B, Brand C and Brand D (n=192 wells).

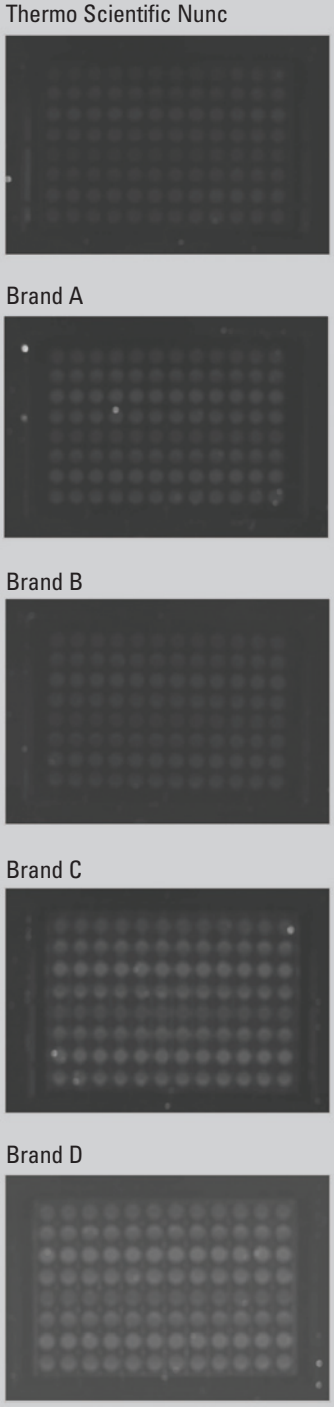

Fig. 2
Auto-fluorescence images of microplates: Thermo Scientific Nunc, Brand A, Brand B, Brand C and Brand D.

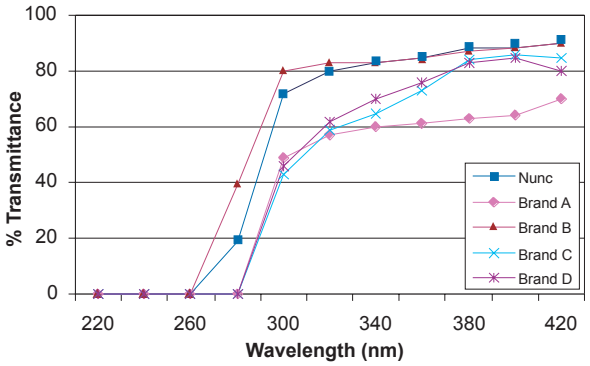

Fig. 3
The % Transmittance of 96 well microplates: Thermo Scientific Nunc, Brand A, Brand B, Brand C and Brand D.

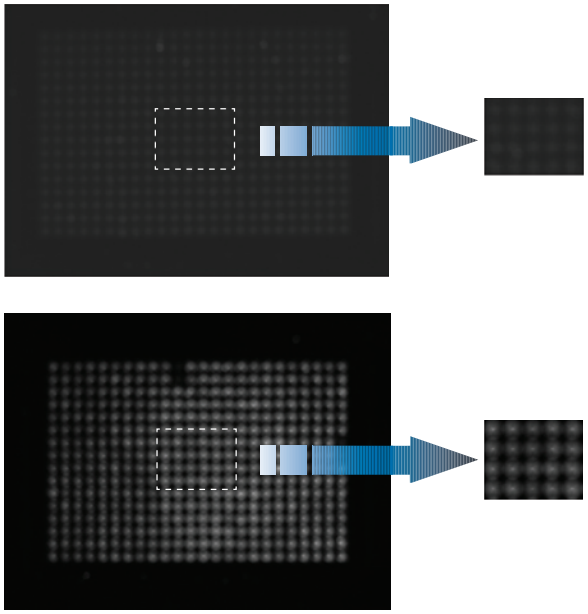

Fig. 4
The fluorescence images of 384 well microplates. Thermo Scientific Nunc plates with a low, homogeneous auto-fluorescence background, and Brand E with significant auto-fluorescent background and fluorescence halo effect.

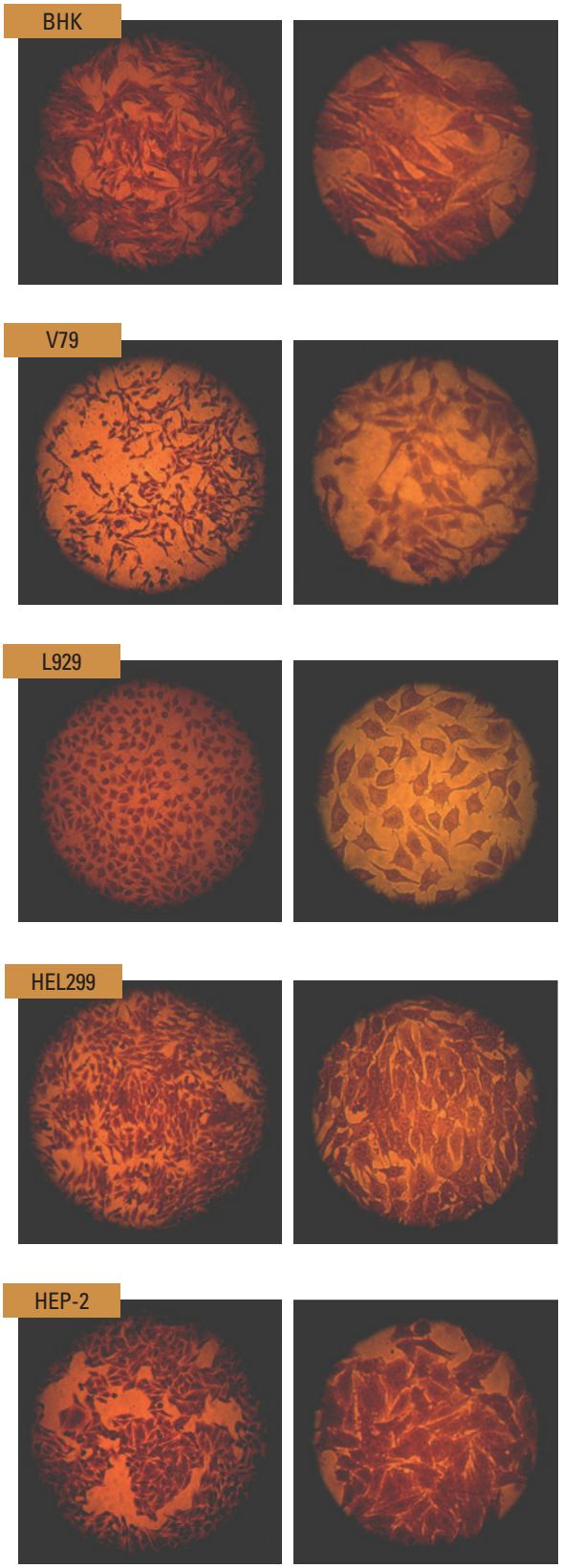

Fig. 5
Light micrographs of cell adhesion (left panel, 100x) and cell morphology (right panel, 250x) of different cell lines: BHK, V79, L929, HEL299 and Hep-2.

Conclusion

The accuracy and reproducibility of an assay depends in large part upon the quality of the polymer substrate used in the construction of the microplate. Thermo Scientific Nunc microplates demonstrated superior properties in terms of transmittance, total fluorescent intensity, low auto-fluorescence and absence of a halo effect when compared to other brands.

Nunc 96 and 384 well black microplate features:

- Low auto-fluorescence background
- No cross-talk between wells
- Optical clarity
- No fluorescence halo effect
- Excellent cell adhesion and cell growth

Thanks to

Fumin Chiu, Tom Cummins, Ron Ringleben, Heidi McIntosh, Joe Granchelli

Coverglass Optical Bottom Plates Specifications

Thermo Scientific Nunc 96 Well Optical Bottom Plates, Polystyrene/Polymer base

Cat. No.	Surface	Color	Total vol. µL/well	Sterile	With lid	Units per pack/case
165305	Cell Culture	White	400	+	+	10/30
165306	Cell Culture	Black	400	+	+	10/30
152028	Poly-D-Lysine	White	400	-*	+	5/20
152036	Collagen I	Black	400	-*	+	5/20
152037	Poly-D-Lysine	Black	400	-*	+	5/20
152040	Collagen I	White	400	-*	+	5/20
265301	Non-treated	Black	400	-	-	10/30
265302	Non-treated	White	400	-	-	10/30

* Produced in clean environment

Thermo Scientific Nunc 96 Well Optical Bottom Plates, Polystyrene/Coverglass

Cat. No.	Surface	Color	Total vol. µL/well	Glass thickness**	Sterile	With lid	Units per pack/case
146518*	Cell Culture	Black	400	1.0	+	+	1/20
160376	CC^2	Black	400	1.5	+	+	6/30
164588	Cell Culture	Black	400	1.5	+	+	6/30
164590	Cell Culture	White	400	1.5	+	+	6/30
265300	Non-treated	Black	400	1.5	-	-	5/30

* Not available in Americas
** No. 1.0 = 0.13-0.16 mm, No. 1.5 = 0.16-0.19 mm

Thermo Scientific Nunc 384 Well Optical Bottom Plates, Polystyrene/Polymer base

Cat. No.	Surface	Color	Total vol. µL/well	Sterile	With lid	Units per pack/case
142761*	Cell Culture	Black	120	+	+	10/30
142762*	Cell Culture	White	120	+	+	10/30
152029	Poly-D-Lysine	Black	120	-**	+	5/20
152041	Collagen I	Black	120	-**	+	5/20
164730*	Cell Culture	Black	120	+	-	10/30
242763	Non-treated	White	120	-	-	10/30
242764	Non-treated	Black	120	-	-	10/30

* Certified
** Produced in clean environment

Thermo Scientific Nunc 384 Well Optical Bottom Plates, Polystyrene/Coverglass

Cat. No.	Surface	Color	Total vol. µL/well	Glass thickness**	Sterile	With lid	Units per pack/case
146508*	Cell Culture	Black	120	1.0	+	+	1/20
164586	Cell Culture	Black	120	1.5	+	+	6/30
240074	Non-treated	Black	120	1.5	-	-	5/30

* Not available in Americas
** No. 1.0 = 0.13-0.16 mm, No. 1.5 = 0.16-0.19 mm

References

1. R.L. Grant, D. Acosta.
   Ratiometric Measurement of Intracellular pH of Cultured Cells with BCECF in a Fluorescence Multi-Well Plate Reader.
   In Vitro Cell Devel Biol Anim 33, 256 (1997)

2. R.P. Wersto, et al.
   Uptake of Fluorescent Dyes Associated with the Functional Expression of the Cystic Fibrosis Transmembrane Conductance Regulator in Epithelial Cells.
   Proc Natl Acad Sci USA 93, 1167 (1996)

3. R. Wronski, N. Golob, E. Grygar, M. Windisch.
   Two-Color, Fluorescence-Based Microplate Assay for Apoptosis Detection.
   BioTechniques 32:666-668 (March 2002)

4. T. Nordström, et al.
   A Microplate-Based Fluorometric Assay for Monitoring Human Cancer Cell Attachment to Cortical Bone.
   Anal Biochem 267, 37 (1999)

5. F.J. Alvarez-Leefmans, J. Altamirano, W.E. Crowe.
   Use of Ion-Selective Microelectrodes and Fluorescent Probes to Measure Cell Volume.
   Meth Neurosci 27, 361 (1995)

6. C.L. Aparicio, L.H. Strong, M.L. Yarmush.
   Correction For Label Leakage In Fluorometric Assays Of Cell Adhesion.
   Short Technical Reports, BioTechniques 23:1056-1062 (Dec 1997)

7. L.S. De Clerck, et al.
   Use of Fluorescent Dyes in the Determination of Adherence of Human Leucocytes to Endothelial Cells and the Effect of Fluorochromes on Cellular Function.
   J Immunol Meth 172, 115 (1994)

8. J.R. Sellers, S. Cook, V.S. Goldmacher.
   A Cytotoxicity Assay Utilizing a Fluorescent Dye That Determines Accurate Surviving Fractions of Cells.
   J Immunol Meth 172, 255 (1994)

9. L.G. Mendoza, P. McQuary, A. Mongan, R. Gangadharan, S. Brignac.
   High-Throughput Microarray-Based Enzyme-Linked Immunosorbent Assay (ELISA).
   Research Reports, BioTechniques 27:778-788 (Oct 1999)

10. J. Bigler, C. Chen, J.D. Potter.
    Determination of Human NAT2 Acetylator Genotype by Oligonucleotide Ligation Assay.
    Short Technical Reports, BioTechniques 22:682-690 (April 1997)

11. J.S. Leeder, et al.
    Fluorescence-Based Viability Assay for Studies of Reactive Drug Intermediates.
    Anal Biochem 177, 364 (1989)

Austria
+43 1 801 40 0

Belgium
+32 53 73 42 41

China
+86 21 68654588

Denmark
+45 4631 2000

France
+33 2 2803 2180

Germany
+49 6184 90 6940

India
+91 22 6716 2200

Italy
+39 02 02 95059 or 434-254-375

Japan
+81 3 3816 3355

Netherlands
+31 76 571 4440

Nordic/Baltic countries
+358 9 329 100

North America
+1 585-586-8800

Russia/CIS
+7 (812) 703 42 15

Spain/Portugal
+34 93 223 09 18

South America
+1 585 899 7298

Switzerland
+41 44 454 12 12

UK/Ireland
+44 870 609 9203

Other Asian countries
+852 2885 4613

Countries not listed
+49 6184 90 6940 or
+33 2 2803 2180

www.thermoscientific.com

TILSPNUNCBU15 0610

nunc

Thermo SCIENTIFIC