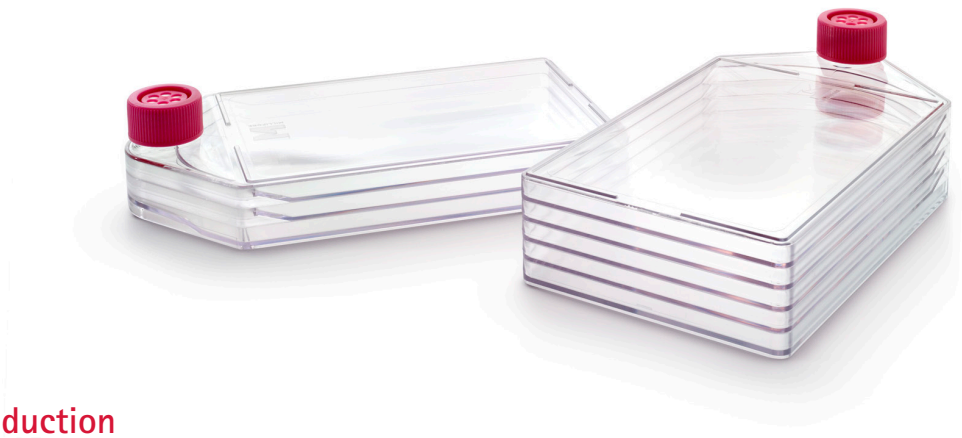
Application Note

Millicell® HY Multilayer Culture Flasks Enable Higher Cell Recovery and Space Savings Compared to Other Multilayer Flasks



Introduction

Increasingly, research studies involve the analysis of multiple culture conditions and simultaneous interrogation of multiple pathways or analytes, requiring larger and larger numbers of cells, especially for systems-level analyses. To meet these demands while not exceeding the fixed limitations of laboratory funds and incubator space, multilayer flasks have become essential cultureware.

Multilayer flasks have increased efficiency in numerous research applications, including cell banking, cell-based assays and virus production by increasing the surface area for cell growth while maintaining a footprint similar to traditional T-flasks. Researchers can culture more cells in the same space and environment by growing cells on multiple layers. Also, multilayer flasks save time and tedium associated with processing multiple single-layer flasks.

Multilayer flasks from different suppliers, however, feature differences in design, geometry, and handling. In this study, we assessed the relationship between flask design and flask performance by comparing Merck Millipore's Millicell® HY 3- and 5-layer flasks to corresponding multilayer flasks from Supplier B (Figure 1).

Among other differences, we found that Millicell® HY Flasks provided higher yields of cells per unit surface area, increased space savings, better pipette access (permitting larger volume pipettes (up to 25 mL)), and easier visualization of cells by microscopy than the flasks from Supplier B.

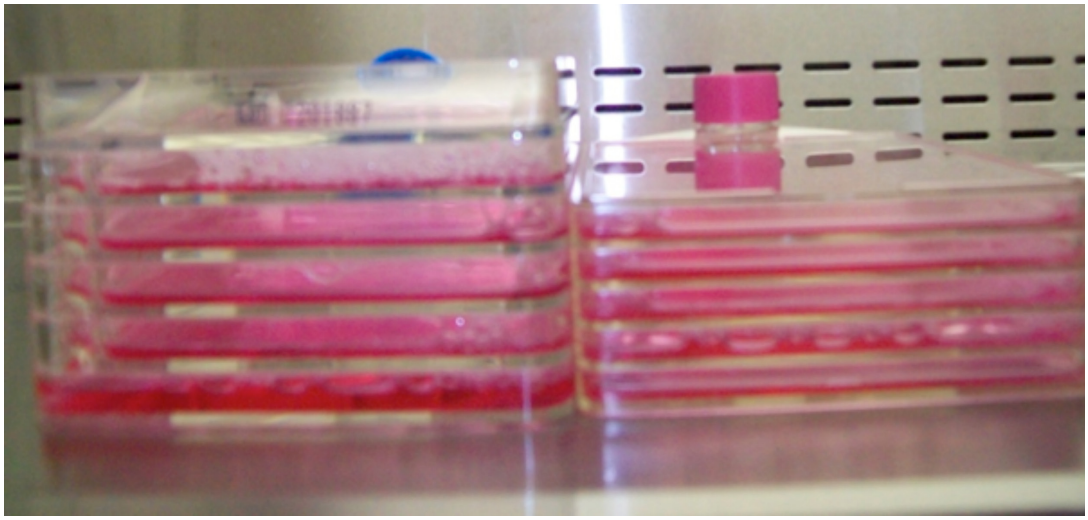


Figure 1.

More space savings. Supplier B's 5-layer flask occupies 1.25 in. (3.18 cm) more vertical space than the Millicell® HY 5-layer flask.

Supplier B's flask (left) is ~3.25 inches and Millicell® HY flask (right) is ~2 inches high.

Materials and Methods

Cells and medium

NIH 3T3 cells were removed from cryostorage and seeded at 4,000 cells/cm^2 in a T75 flask in Dulbecco's Eagle Modified Medium (DMEM, Fisher Scientific Cat. No. SH30022.01), 10% heat-inactivated fetal bovine serum (FBS, Hyclone Laboratories Cat. No. SH30071), Ciprofloxacin (Fisher Scientific Cat. No. NC9553331) at 37 °C, 5% CO_2.

Medium requirements

For both Millicell® HY flasks and flasks from Supplier B, we used 0.2 mL medium per cm^2 flask surface area. Since the Millicell® HY flasks have greater surface area than Supplier B flasks, 40 mL of medium was required per layer. We used 35 mL medium per layer in flasks from Supplier B.

Subcultivation

Cells were allowed to reach up to 80% confluency before being subcultured as needed. Subcultivation ratio used was 2 to 3 X 10^3 cells/cm^2. Cells were subcultured by removing medium, washing with HBSS (25 mL per flask layer), adding 0.04 mL/cm^2 of trypsin-EDTA (Fisher Scientific Cat. No. SH30236.01), then adding 8 mL of complete medium per layer.

Cell harvesting

70% confluent cell monolayers were trypsinized and suspended in complete medium. Cells were then pelleted, supernatant removed, and pellets resuspended in 20 mL Hank's Balanced Salt Solution (HBSS, Fisher Scientific Cat. No. SH30031.02) (total) for 3-layer flasks and 40 mL HBSS (total) for 5-layer flasks.

Cell counting and viability determination

For cell counting and viability analysis, ViaCount® Flex Reagent (Merck Millipore Cat. No. 4000-00110) was diluted 1:2 with dimethylsulfoxide (DMSO, Fisher Scientific Cat. No. 1281) and mixed well to make a 0.5X solution of ViaCount® Flex Reagent. Cell samples were mixed by vortexing, and then 200 µL of cell suspension was transferred to a 1.5 mL microcentrifuge tube and 2 µL of the 0.5X ViaCount® Flex reagent was added. The sample was mixed until a homogenous pink color appeared, and no dark purple reagent was visible. The sample was incubated for 5 minutes at room temperature for dye equilibration, and then the sample was analyzed on a guava easyCyte™ benchtop flow cytometer (Merck Millipore).

Results

Comparing overall handling of the two types of flasks showed some differences (Table 1). The ability of the Millicell® HY Flasks to allow access to 25 mL pipettes may, in part, explain the higher cell recovery exhibited by these flasks.

The increased height of the 5-layer flasks from Supplier B makes it difficult to observe cells under a microscope, especially if the stage cannot be lowered.

On average, Millicell® HY 3- and 5-layer flasks yielded 600 cells/cm^2 more than 3- and 5-layer flasks from Supplier B (Figure 2).

	Millicell® HY Flasks		Supplier B Multilayer Flasks	
	600 cm^2	1000 cm^2	525 cm^2	875 cm^2
Cell viability	+++	+++	+++	+++
Liquid Mixing	+++	+++	+++	+++
Pipette Access	+++	+++	++ [1]	++ [1]
Transfer of liquid (pouring)	++ [2]	++ [2]	+++	+++
Stacking	+++	+++	+++	+++
Viewing under microscope to allow qualitative analysis	+++	+++	+++	+ [3]
Labeling flasks	+++	+++	+++	+++
Upon Harvesting of Cells:				
% Confluency	~70%	~70%	~70%	~70%
% Viability	97%	92%	95%	92%
Yield (Cells/cm^2)	3380.0	3413.0	2777.0	2836.0

[1] Millicell® HY Flasks pipette access allows for up to 25 mL size; Supplier B flasks allow up to 10 mL.
[2] Pouring must be done slowly to prevent splashing.
[3] Observation due to overall flask size or fit; size is awkward.
Depending on microscope used, viewing may not be possible if microscope cannot be adjusted.
(+++ = Good, ++ = Fair, + = Poor)

Table 1.
Comparison of multilayer flask features.

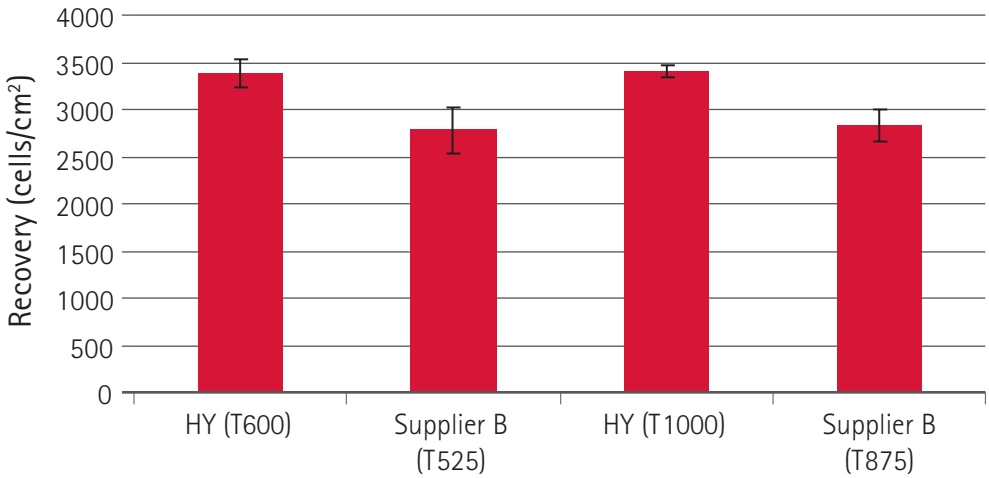


Figure 2.
Higher recovery. On average, Millicell® HY Flasks yielded 600 cells/cm^2 more than corresponding flasks from Supplier B. Error bars represent standard deviation.

Conclusions

Although many suppliers of cell cultureware have introduced multilayer flasks on the market, we have shown that differences in flask design can greatly impact cell yield. By yielding more cells per unit surface area, Millicell® HY Flasks enable users to use less culture medium and fewer flasks when generating large numbers of cells for their experiments. Also, by taking up less space in an incubator, these flasks enable laboratories to accommodate larger-scale experiments and/or larger numbers of researchers to share the same space. Choosing the right multilayer flask can give any laboratory the power to ask research questions of larger scope, even with limited resources.

Ordering Information

Description	Catalogue No.
Millicell® HY 3-Layer Cell Culture Flask, T600, sterile	PFHYS0616
Millicell® HY 5-Layer Cell Culture Flask, T1000, sterile	PFHYS1008
guava ViaCount® Kit	4500-0110
guava easyCyte™ Flow Cytometer	0500-4008 and others

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