



Content

1. Key Facts
2. Abstract
3. High Content Screening
4. High Throughput Screening
5. Cycloolefins as a Base Material for Microplates
6. Features and Advantages of SCREENSTAR Microplates
7. Ordering Information
8. Literature

SCREENSTAR:

A new 1536 Well Microplate for High Content and High Throughput Screening

1. Key Facts

- Cycloolefin film bottom with excellent optical properties for glass-like image quality and excellent resolution
- Thin film bottom (190 µm) compatible with specifications of commercially used microscopic objectives for perfected focusing
- Recessed well bottoms enable low working distances and complete periphery access in high-resolution microscopy with oil or water immersion objectives
- Cell culture treatment and sterility (SAL10⁻³) assures exceptional performance in cell-based assays
- Reduced autofluorescence and absorption in the lower UV for superior data quality in biochemical assays
- Dimensions conform with ANSI recommendations for ease of automation
- Smooth microplate top absent of alphanumeric coding facilitates flush lid mounting for use within ultra high throughput screening systems
- SCREENSTAR microplates are shrink-wrapped in recyclable PET bags with a bottom tray enclosure for added protection of the film bottom

2. Abstract

SCREENSTAR is a new generation microplate for advanced high content and high throughput screening. Manufactured entirely of cycloolefin, an innovative resin with advanced optical properties, the SCREENSTAR microplate features a black pigmented frame with a 190 μm ultra-clear film bottom. Combining excellent optical features with high-quality surfaces for cell culture and biochemical assays, the microplate design facilitates use of high magnification objectives with a low working distance, ideally suited for fully automated screening systems.

3. High Content Screening

High content screening (HCS), or the microscopic analysis of induced cell reactions (Fig. 1), is one of the most important tools in advanced drug discovery [1, 2].

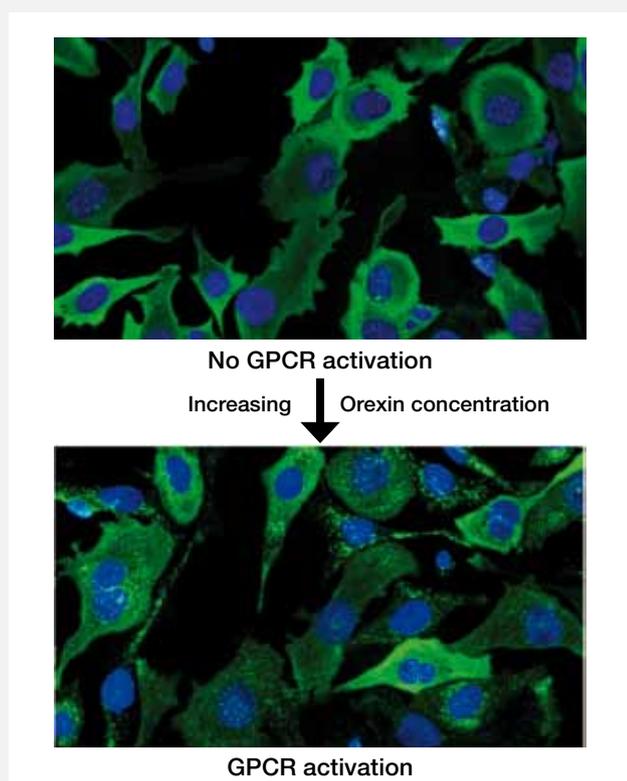


Figure 1: Example of high content screening: Translocation of β -Arrestin after G-Protein Coupled Receptor activation in 1536 well SCREENSTAR microplates (Pathhunter, DiscoverX Corporation, # 93-0258E2C1, Fremont, USA / ImageXpress[®] Micro Widefield HCS System, 20x, Molecular Devices, Sunnyvale, California, USA). The excellent optical properties of the thin film bottom lead to outstanding image quality with optical low background and high resolution.

In contrast to classical high throughput screening based on biochemical assays, HCS permits the observation of cell behaviour at the cellular level. High content screening can reveal cell reactions which are not detected when concentrating on single enzyme-catalysed reactions. A variety of parameters can influence the success of high content screening. Important factors in HCS are the microscopic system, the optical features of the microscopic objective such as the numerical aperture, the software tools implemented for data mining and analysis, the specimen itself and the substrate or surface used to contain it (i.e. microplate).

4. High Throughput Screening

Classical high throughput screening based on biochemical or cell-based assays (Fig. 2) with a subsequent readout in a plate reader or plate imager continues to be one of the most popular tools used in drug discovery. The ease of automation [3] for this well-established approach, combined with rapid data analyses and interpretation, provides a highly appropriate primary screening strategy with high turnover of tested compounds.

Although there is a large variety of different microplates available for high throughput screening, investigators continually seek improvements for HTS assay sensitivity and reliability.

The new SCREENSTAR microplate offers an optimal new tool for classical HTS assays in that it allows sensitive signal detection with fluorescence and transmission measurements. Advanced optical properties, low autofluorescence and low UV light absorption render SCREENSTAR microplates especially suitable for signal detection in the lower UV.

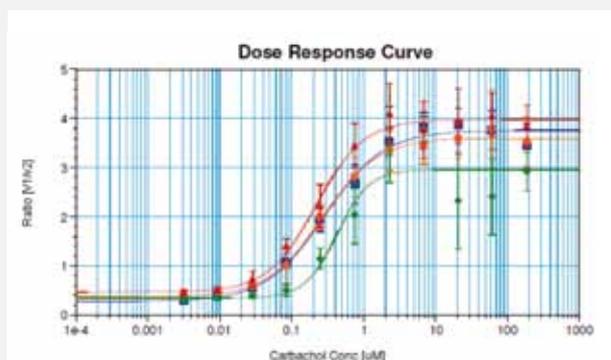


Plate format: 1536 well
Spectramax® Paradigm®, Molecular Devices, Sunnyvale, CA, USA
Cartridge: FI-FRET
● 1536 Well SensoPlate™
● 1536 Well Competitor Microplate
● 1536 Well SCREENSTAR Microplate
● 1536 Well Polystyrene Microplate (µClear®)

Figure 2: Example of a classical high throughput screening assay: Dose response curve based on G-Protein activation. (Geneblazer® beta lactamase reporter gene assay, # 0200-7006, Invitrogen, Carlsbad, USA). Carbachol was used for activation of the muscarinic G-protein. The readout was performed at an excitation wavelength of 405 nm. G protein activation was measured at emission: 520 nm / 447 nm

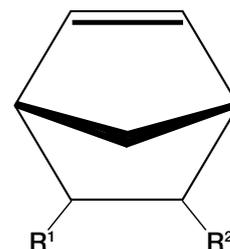


Figure 3: Structural formula of Norbornene: Monomer of cycloolefins

Properties of Cycloolefins

- Amorphous polymers
- Glass-like properties
- Refractive index $n = 1.533$
- Low autofluorescence background in the lower UV
- High moisture barrier with low water absorption rate
- High purity (low extractable)

5. Cycloolefins as a Base Material for Microplates

In addition to polystyrene and glass, cycloolefins (Fig. 3) have become increasingly popular for use as the base material for microplate manufacturers. Cyclic olefin polymers comprise a new class of polymeric materials with inherent physical properties of high glass transition temperature, optical clarity, low shrinkage, low moisture absorption, and low birefringence [4]. Cycloolefins are frequently used to manufacture compound storage microplates [5] due to their low water absorption, high vapour barrier, low level of leachables and resistance to DMSO, the most prevalent compound solvent in high throughput screening.

Cycloolefins also possess excellent optical features. They display a low background in the lower UV with a refractive index and focus background comparable to glass (Fig. 4). [A summary of the chemical, physical and optical properties of cycloolefins is given in the technical appendix of the Greiner Bio-One product catalogue.]

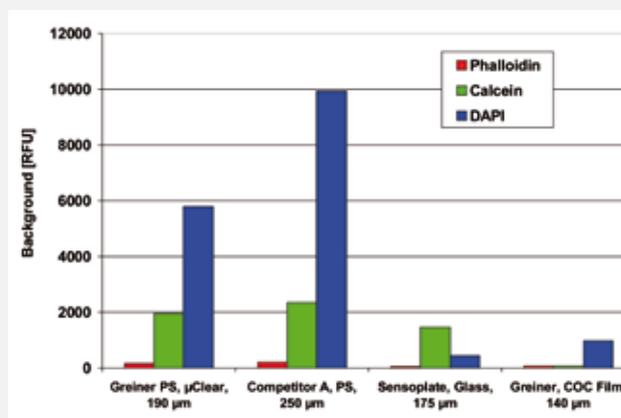


Figure 4: Background of different clear bottom microplates. The bottom thickness and the bottom material have a significant impact on autofluorescence, especially in the lower UV.

DAPI: 358 nm / 461 nm
Phalloidin Tritc: 557 nm / 576 nm
Calcein: 495 nm / 515 nm

6. Features and Advantages of SCREENSTAR Microplates

To meet the advanced requirements of high content screening and drug discovery, the SCREENSTAR microplate product range was created with the development of a new 1536 well microplate.

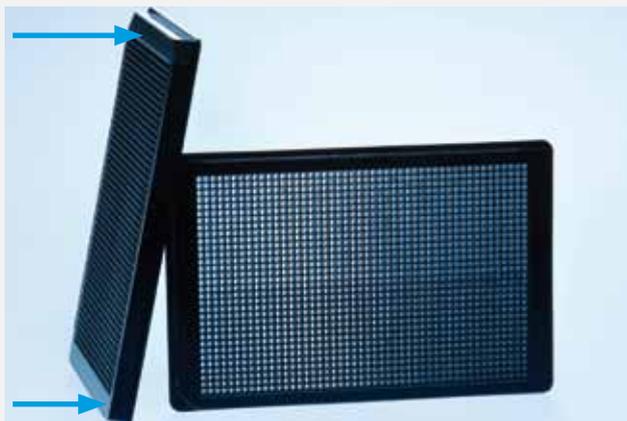


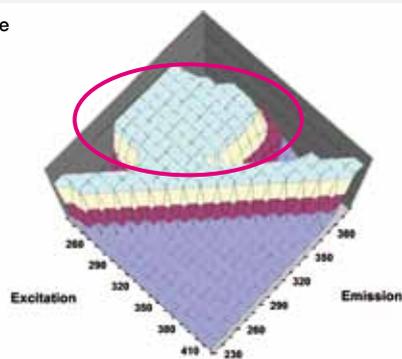
Figure 5: 1536 well SCREENSTAR microplate manufactured entirely of cycloolefin, featuring a black pigmented frame with a 190 µm ultra clear film bottom. A smooth microplate top absent of alphanumeric coding facilitates flush lid mounting for use within ultra high throughput screening systems.

6.1 Optics

The 1536 well SCREENSTAR microplate is manufactured entirely of cycloolefin, featuring a black pigmented frame with a 190 µm ultra clear film bottom (Fig. 5) for ideal compatibility with instrument optics. The well bottom displays excellent optical properties comparable to glass for the highest optical transparency with reduced autofluorescence in the lower UV range, low birefringence and a refractive index of 1.53 (Fig. 4, 6, 7, 8).

Autofluorescence: 190 µm film bottom

Polystyrene



Cycloolefin

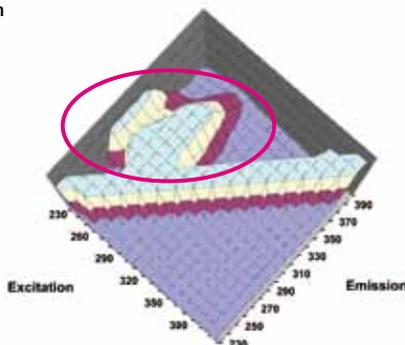


Figure 6: Autofluorescence in the lower UV of cycloolefin compared to polystyrene. Due to the aromatic ring structure, polystyrene demonstrates increased autofluorescence below 400 nm

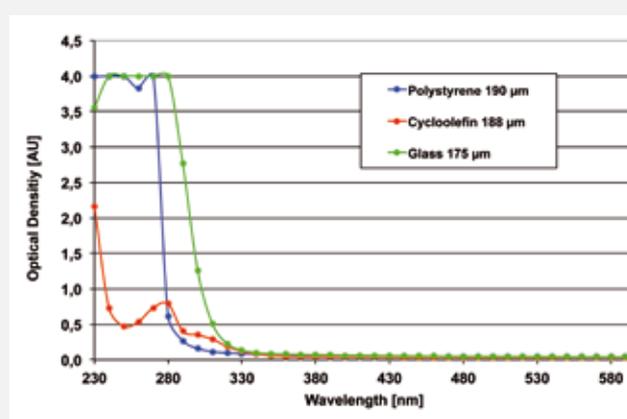
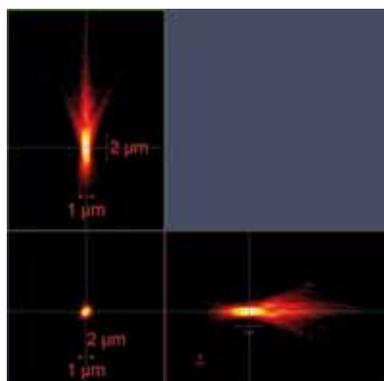


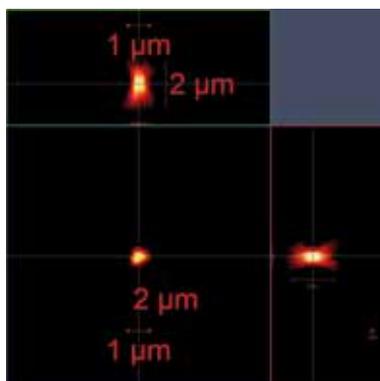
Figure 7: UV absorption scan from 230 nm to 600 nm. Concomitant with low autofluorescence, cycloolefins exhibit very low light absorption, especially in the UV range below 400 nm.

Out of Focus Blur of Different Materials

Polystyrene 190 μm



Glass 175 μm



Cycloolefin 190 μm

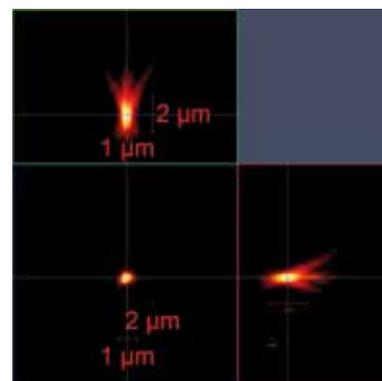


Figure 8: Confocal imaging (LSM 710, 63x, oil immersion, Zeiss AG, Göttingen, Germany) of fluorescent beads in microplates with different film bottoms. Images display the out-of-focus blur obtained with different materials (polystyrene, glass and cycloolefin). The cycloolefin image is more comparable to glass.

6.2 Well Bottom Design

The recessed microplate wells (Fig. 9) enable complete periphery access for high magnification (Fig. 10) and short working distances for oil or water immersion lenses objectives leading to a higher resolution and higher image quality due to the increase in the refractive index of the optical system.

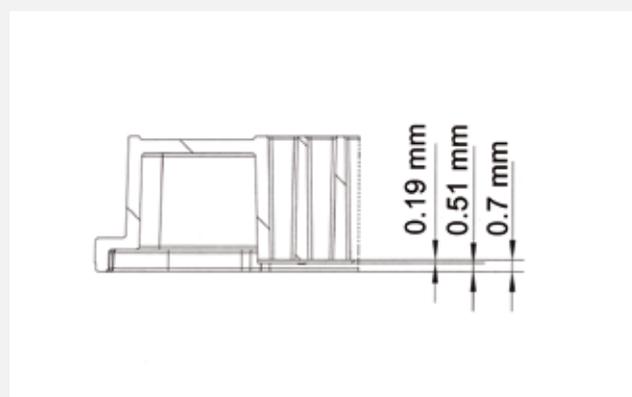


Figure 9: Recessed well bottom of the 1536 well SCREENSTAR microplate. The distance between plate rim and inner well bottom is 0.7 mm. The distance between well rim and outer well bottom is 0.51 mm. The film bottom thickness is 190 μm .

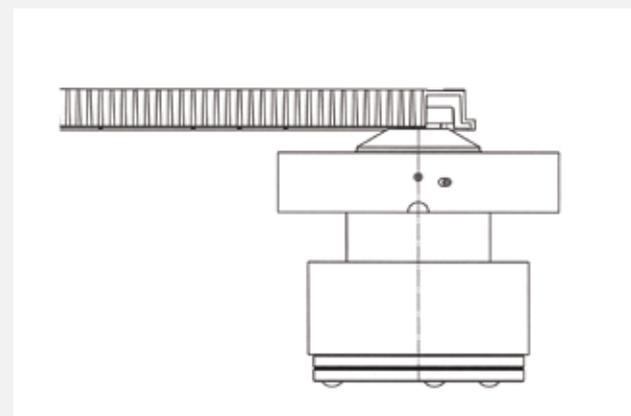


Figure 10: A basic requirement for the new SCREENSTAR 1536 well microplate was the compatibility to high magnification objectives such as oil and water immersion lenses. These objectives are almost touching the microplate bottom, separated just by a thin oil or water layer. For this technology, the microplate bottom must be close to the microplate rim (Fig. 9) to easily allow interchange of microscopic objectives and complete access of the peripheral wells.

6.3 Image Quality

Microscopic objectives are typically calibrated to a 175 µm film bottom thickness, the standard thickness of coverslip glass. Because the tolerances of coverslip glass are +/-20 µm, the ideal bottom thickness of microplates for high content screening should be in the range of 155 to 195 µm.

With a 190 µm film bottom, the 1536 well SCREENSTAR microplate is perfectly suited for the optical requirements of most microscopic systems, resulting in excellent image quality with high resolution (Fig. 11 and 12).

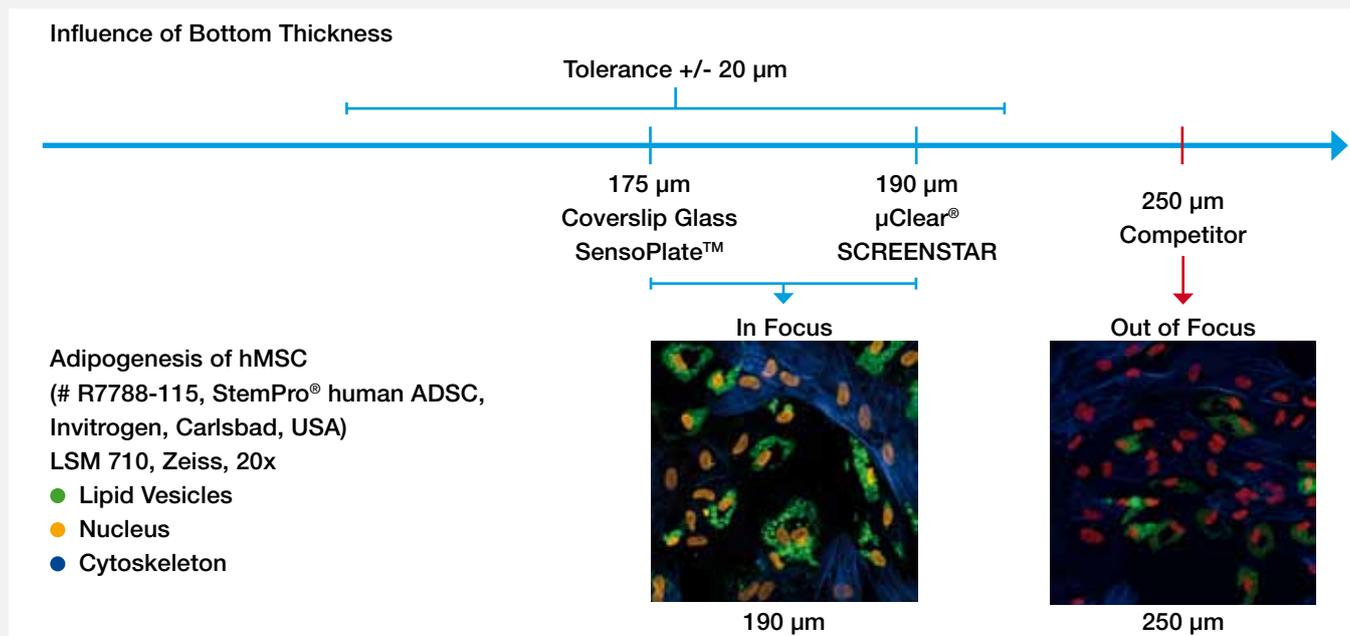


Figure 11: Influence of bottom thickness on resolution and image quality. The adipogenesis of human mesenchymal stem cells (hMSC) was analysed in microplates with 190 µm film bottom and competitive microplates with 250 µm film bottom. The 190 µm film bottom demonstrates exceptional performance, giving high image quality with excellent resolution due to the bottom thickness being within the tolerance range of the microscopic system (Operetta® High Content Imaging System, PerkinElmer, Waltham, Massachusetts, USA). The 250 µm film bottom of the competitive microplate yield blurry images, incapable of sharp focus and magnifications above 20x.

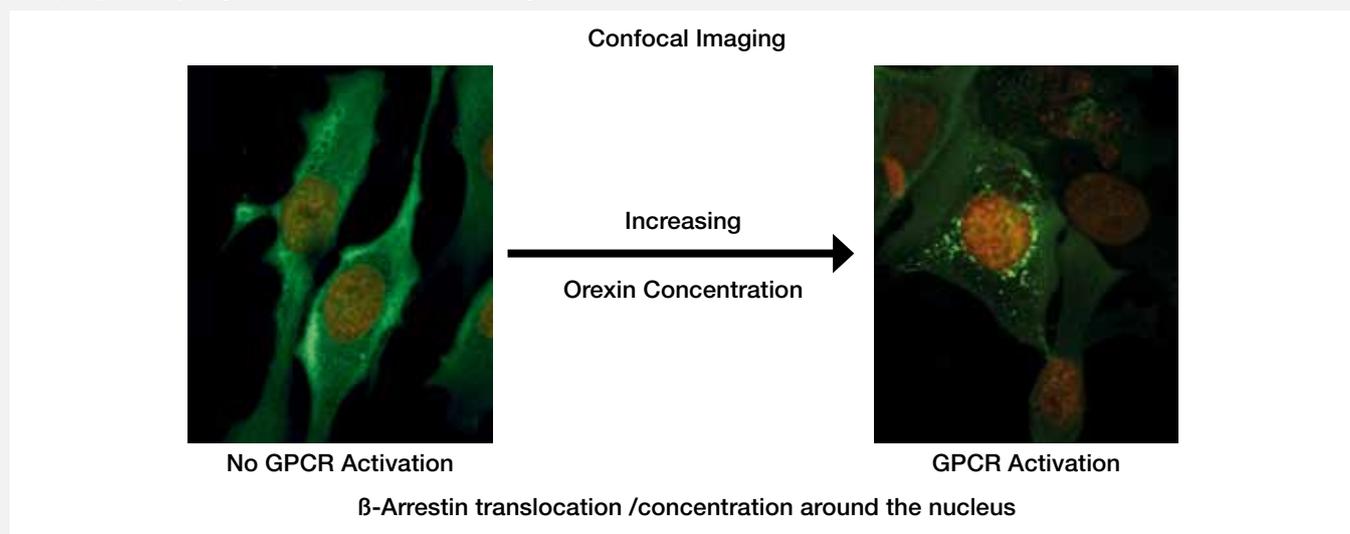


Figure 12: High resolution, confocal microscopy (LSM 710, 63x, oil immersion, Zeiss AG, Göttingen, Germany) in 1536 well SCREENSTAR microplates. Translocation of β-Arrestin after G-Protein Coupled Receptor activation (Pathhunter, DiscoverRx Corporation, # 93-0258E2C1, Fremont, USA) in 1536 well SCREENSTAR microplates.

6.4 Dimensions and Tolerances / Adaptation to Automated Systems

All major dimensions of the 1536 well SCREENSTAR microplate follow ANSI recommendations (ANSI 1-2004) to facilitate adaptation and use in fully automated systems (Fig. 13 and 14). A total plate height of 8 mm overcomes the high/low base design strategy of standard polystyrene clear bottom 1536 well microplates to enable ease of use in dispensing devices without movement of the liquid handler in the Z position. The recessed well bottom (Fig. 9) facilitates access for optical systems, and a smooth microplate top, absent of alphanumeric coding, assists flush lid mounting for use within ultra high throughput screening systems.

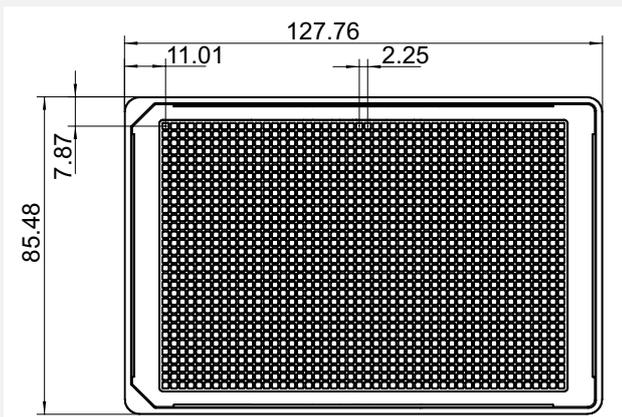


Figure 13: Plate view from top

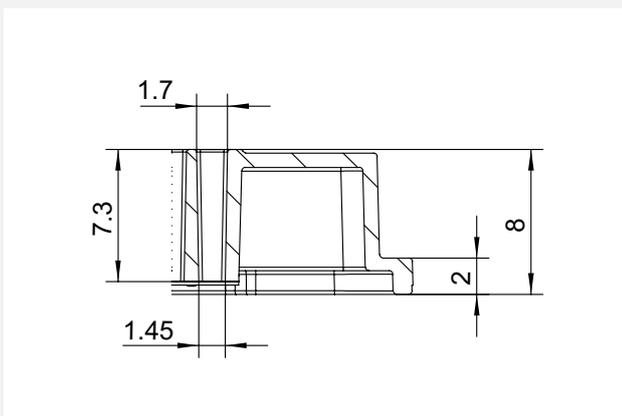


Figure 14: Well profile 1536 well SCREENSTAR microplate
 Total volume: 17.8 μ l
 Working volume: 3 - 15 μ l
 Growth area: 2.1 mm²

6.5 Surface Chemistries

The SCREENSTAR microplates are available sterile with a hydrophilic cell culture treated surface for cell-based assays and non-treated with a hydrophobic surface for biochemical assays. Homogenous cell attachment and cell growth is essential for high Z-factors and reliable results in cell-based high content or high throughput screening. Due to the polymeric structure of cycloolefins, the classical CELLSTAR[®] cell culture treatment is effective in promoting outstanding cell attachment and cell growth (Fig. 15). Thus the SCREENSTAR microplate is the perfect combination of excellent glass-like optical properties with an outstanding cell culture surface. Biological coatings, Advanced TC[™] polymer modification or high binding treatment for SCREENSTAR microplates are available on request. Further ordering information is given in chapter 7.

Cell Attachment and Cell Growth

HeLa, Crystal violet,
 Leica DM IL Fluo / 10x

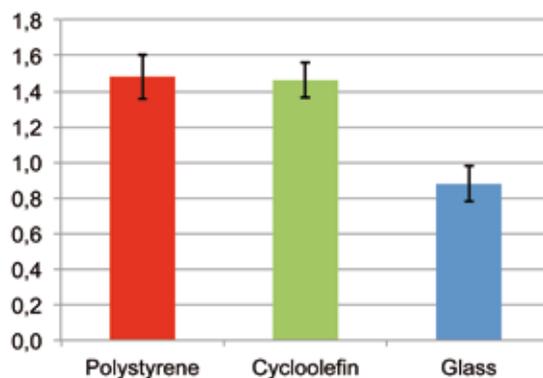
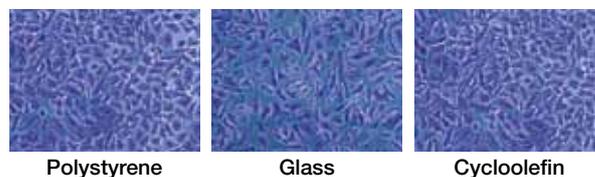


Figure 15: Cell attachment and cell growth of HeLa cells on different surfaces. Cell growth on CELLSTAR[®] cell culture treated cycloolefin surfaces is comparable to polystyrene. SCREENSTAR microplates feature excellent optical and cell culture properties.

6.6 Lid

The 1536 well SCREENSTAR microplates are not supplied with a lid. The ultra low profile lid (Cat. No. 691 161) is recommended for compatible use, as it allows both ease of plate gripping and side barcode detection due to its recessed rim (Fig. 16). Further ordering information is given in the table below.

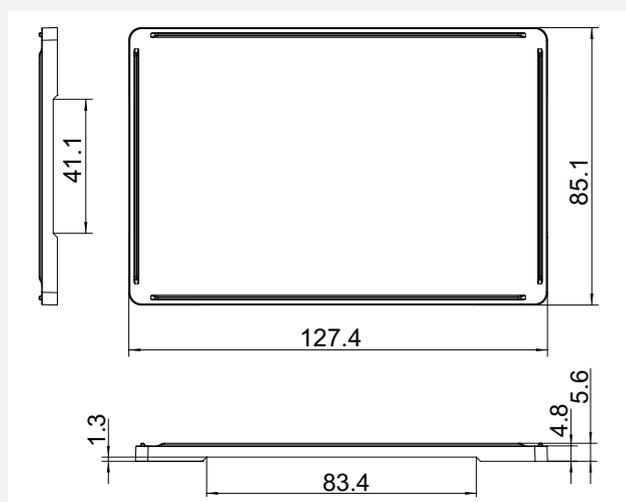


Figure 16: Ultra low profile lid allows easy gripping of the plate and easy barcode detection due to its recessed rim

6.7 Packaging

The plates are shrink-wrapped in easy-open plastic bags within the carton. Additionally, the lowest microplate in a stack of 17 plates is contained within an easy to remove clear plastic tray made of PET to protect the SCREENSTAR μ Clear[®] film bottom of the lowest microplate in the stack.

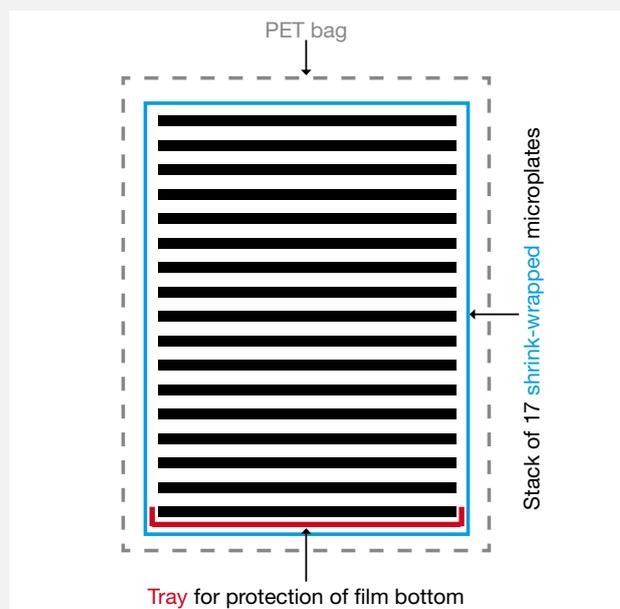


Figure 17: Packaging of 1536 well SCREENSTAR microplates

7. Ordering Information

Cat.-No.	Description	Quantity per bag	Quantity per case
789 866	1536 well SCREENSTAR microplate, cycloolefin, black, 190 μ m μ Clear [®] film bottom, cell culture treated, sterile, without lid	17	68
789 896	1536 well SCREENSTAR microplate, cycloolefin, black, 190 μ m μ Clear [®] film bottom, non-treated, non-sterile, without lid	17	68
691 161	Ultra low profile lid, sterile	5	100
691 101	Ultra low profile lid, non-treated, non-sterile	5	100

8. Literature

- 1) High Throughput Screening, high content screening, primary and stem cells, New techniques now converging, Richard M. Eglen, Drug Discovery World, Spring 2009, pp. 25-31
- 2) Image-based High-content Screening – A view from Basic Science, Peter Lipp & Lars Kaestner, High-Throughput Screening in Drug Discovery, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2006, pp. 129-149
- 3) Tools and Technologies that Facilitate Automated Screening, John Comley, High-Throughput Screening in Drug Discovery, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2006, pp. 37-73
- 4) Chemical Structure and Physical Properties of Cyclic Olefin Copolymers. IUPAC Technical Report. Ju Yong Shin et al. Pure Appl. Chem. Vol. 77, No. 5 pp 801-814, 2005
- 5) Greiner Catalogue 2012/13 Chapter 2 / pp. 29-30

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