N° 16

FORUM

TECHNICAL NOTES & APPLICATIONS FOR LABORATORY WORK

96 WELL HALF AREA MICROPLATES AND THEIR APPLICATION IN FLUORESCENCE, LUMINESCENCE AND TRANSMISSION MEASUREMENTS

CONTENT

1/ INTRODUCTION

Standard 96 well microplates are frequently used for many applications in diagnostics, basic research and the pharmaceutical industry. The 96 well platform offers significant advantages for ease of handling on a manual, semi- and fully automated basis. Manual handling can easily be performed using multichannel pipettes, and multiple varied devices such as microplate readers, washers, and liquid handlers compatible with 96 well microplates are widely used as standard laboratory equipment.

Nevertheless, 96 well microplates feature a rather large well volume, which can become disadvantageous when rare or expensive components are involved in an application. One option to reduce sample volume is to transition to a higher-format 384 or 1536 well microplate, however, higher density formats do not offer the same ease of manual handling found with 96 well microplates. Additionally, not all laboratory equipment can handle both 96 well and high-format plates without significant modification.

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For conservation of volume and materials, 96 well half area microplates offer an interesting alternative. With a well diameter of only 5 mm, in comparison to 7 mm in standard 96 well plates **(Fig. 1),** half area microplates allow up to a 50 % sample volume reduction. Because both the outer dimensions and well center positions are identical to those of a standard 96 well microplate, most devices are compatible with half area microplates without need for special adaptation. Furthermore, manual handling of the plates is as comfortable as with standard 96 well microplates.

The use of half area microplates is described in the literature for a wide range of applications:

- **Spectroscopy, especially DNA, RNA and protein measurements**
- **Biochemical or cell-based fluorescence and luminescence assays**
- **Cultivation of cells and microorganisms**

This Forum outlines examples where the usage of half area microplates is especially advantageous.

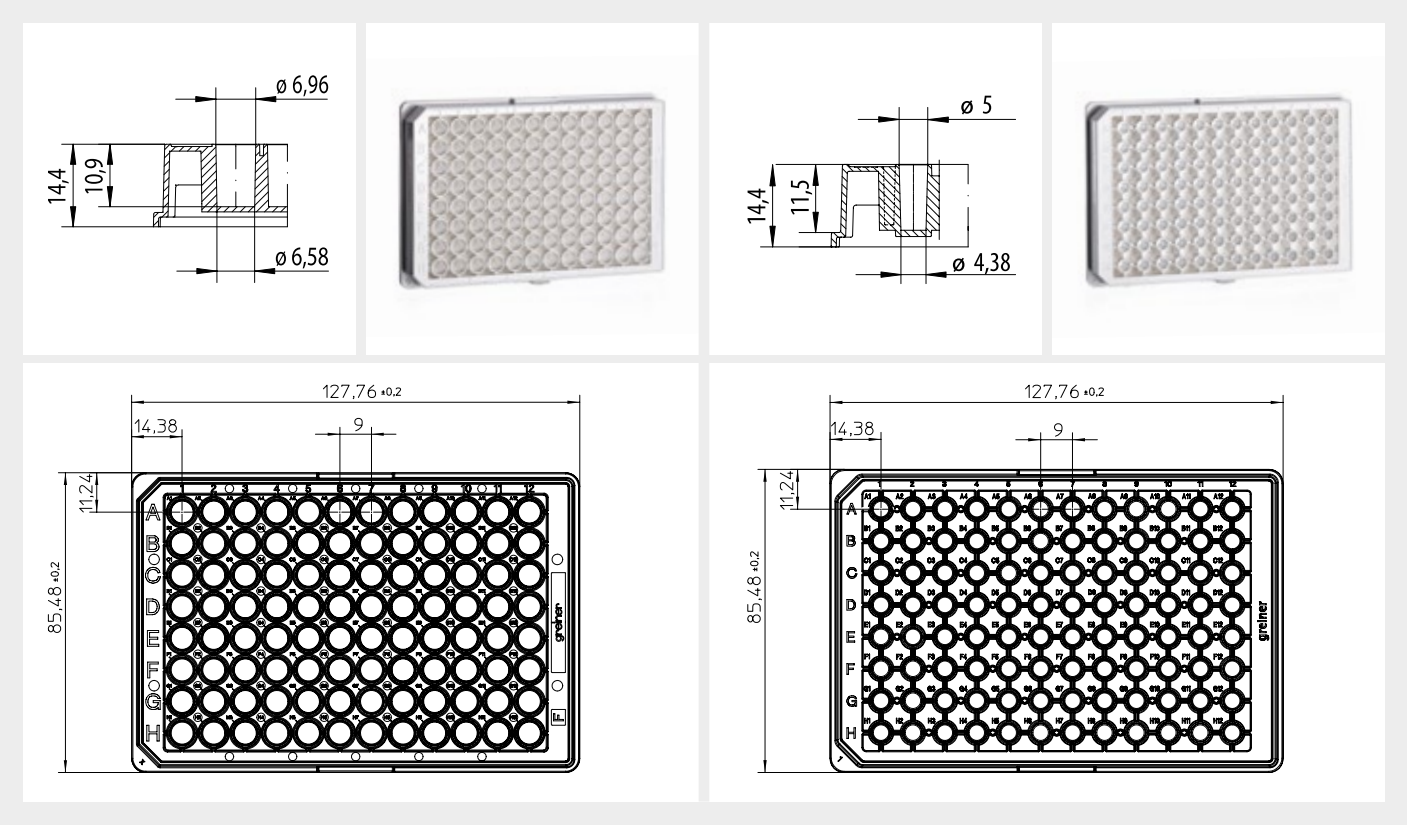

Figure 1a: Well profile and plate information for 96 chimney well microplates

Figure 1b: Well profile and plate information for 96 well half area microplates

Footprint	127.76 x 85.48 mm
A1 row offset	11.24 mm
A1 column offset	14.38 mm
Well spacing (center to center)	9.0 mm
Range or skirt height	2.5 mm
Mathematical volume	392 µl
Working volume	25 - 340 µl
Growth area	34 mm^2

Footprint	127.76 x 85.48 mm
A1 row offset	11.24 mm
A1 column offset	14.38 mm
Well spacing (center to center)	9.0 mm
Range or skirt height	2.5 mm
Mathematical volume	199 µl
Working volume	15 - 175 µl
Growth area	15 mm^2

2/ EXEMPLARY APPLICATIONS FOR HALF AREA MICROPLATES

2.1/ UV/VIS SPECTROSCOPY IN HALF AREA MICROPLATES

UV/VIS spectroscopy plays an important role for many biochemical assays and measurements. For some when rare or expensive substances are used, reduction of sample volume without loss of signal quality is an important aspect. Therefore the use of the half area format can provide a significant advantage for this purpose **[1]**. If spectroscopic measurements in standard and half area 96 well microplates are to be compared, the height of liquid sample in each well has to be taken

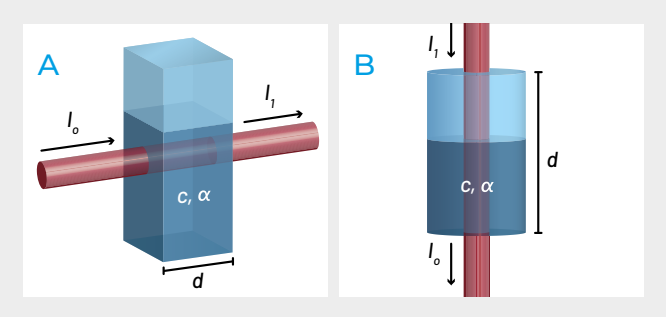

Figure 2: The absorption of light in the visible or UV range is a physical process where the amount of absorbed light depends on the concentration of the substance c, the thickness of liquid layer d and a specific absorption coefficient α at a defined wavelength λ. In a cuvette (A) the thickness of the liquid layer is fixed. In a microplate (B) the thickness of the liquid layer depends to the volume.

into account **(Fig. 2) [2]**. For ease of spectrophotometric measurement and corresponding concentration determinations, the half area microplates have been designed to feature standardised path lengths of 0.5 cm and 1.0 cm with well filling volumes of 80 µl and 170 µl, respectively **(Fig. 3)**.

2.1.1/ IMMUNO ASSAYS (ELISA)

ELISA (Enzyme-Linked Immunosorbent Assay) is one of the most widely used techniques in both basic immunology research and diagnostic analyses. Because ELISA

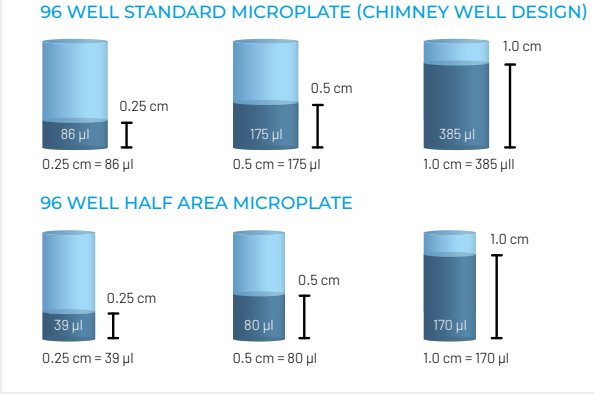

Figure 3: Standardised path length in standard 96 chimney well and half area microplates

enables peptides, proteins, antibodies and hormones to be selectively detected in small concentrations among a multitude of other substances with relatively low cost and high simplicity, the method provides an important and useful tool for disease monitoring, diagnostics and doping tests, as well as environmental and food analytics. ELISA methods yield both sensitive and accurate results, and employment of automated handling with a microplate platform allows rapid conduction of tests in a high-throughput manner. Various detection methods can be utilised for ELISA. Beside those that employ fluorescence- and luminescence-based techniques, the most common method is colorimetric detection based on UV/VIS spectroscopy. In **Figure 4**, a typical direct ELISA application is described.

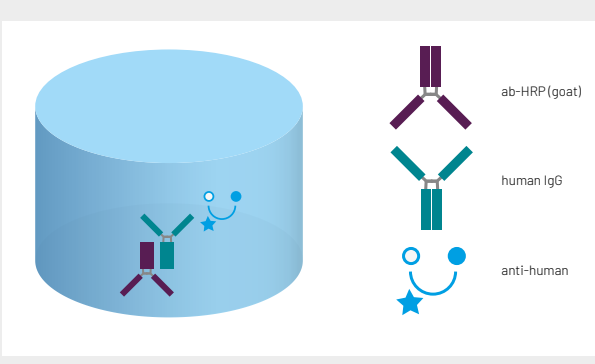

Figure 4: IgG ELISA human IgG (5 µg/ml in carbonate buffer, pH 9.6) is coated to the surface of high-binding microplates. An anti-human antibody horseradish peroxidase conjugate (3.3 ng/ml in PBS-T) is employed for detection with TMB [3].

	96 Well Standard	96 Well Half Area	Reagent Conservation
Covered surface	92 mm²	82 mm²	•
Corresponding liquid height	3 mm	4.7 mm	•
Volume coating solution	100 µl	75 µl	25 %
Washing buffer	350 µl	150 µl	57 %
Anti human IgG-HRP-conjugate	100 µl	75 µl	25 %
OD[1]	1.2	1.3	•

Table 1: Comparison standard - half area microplates 1) After adaptation to 1 cm path length

Detection	Plate color	Surface Properties	Brand Name
Colorimetric	transparent	High-binding Medium-binding	MICROLON 600 MICROLON 200
Fluorescence	black	High-binding Medium-binding	FLUOTRAC 600 FLUOTRAC 200
Luminescence	white	High-binding Medium-binding	LUMITRAC 600 LUMITRAC 200

Table 2: Half area microplates for immuno assays

Table 1 shows the comparison of reagent consumption in a 96 well standard microplate compared to a 96 well half area microplate. A 25 % reduction of coating and antigen solution used for a direct IgG ELISA within half area microplates resulted in optical density values comparable to a standard 96 well microplate. It was possible to even further reduce the amount of washing buffer per well to 60 % of the original standard 96 well volume with similar result.

A key step in an ELISA is the binding of one assay component to the microplate surface. Therefore, the features of this surface are often crucial for the performance of an assay. Half area microplates for ELISA applications are available from Greiner Bio-One with a high-binding and a medium-binding surface as transparent, black or white plates for colorimetric, fluorescence or luminescence detection, respectively **(Table 2)**.

2.1.2/ UV SPECTROSCOPY FOR DETERMINATION OF NUCLEIC ACIDS AND PROTEINS

UV-spectroscopy is a classic analytical method for the determination of nucleic acids and protein concentrations. Absorbance readings for nucleic acid quantifications are generally performed in the lower UV at 260/280 nm, a wavelength range where standard polystyrene microplates are not capable of transmission because of the high adsorption of polystyrene in the lower UV. For lower UV spectrophotometry, UV-Star® microplates feature a combination of a UV-transparent material and a thin film bottom (135 µm), resulting in a microplate with almost no background adsorption in the relevant wavelength range **(Fig. 5)**. The standard

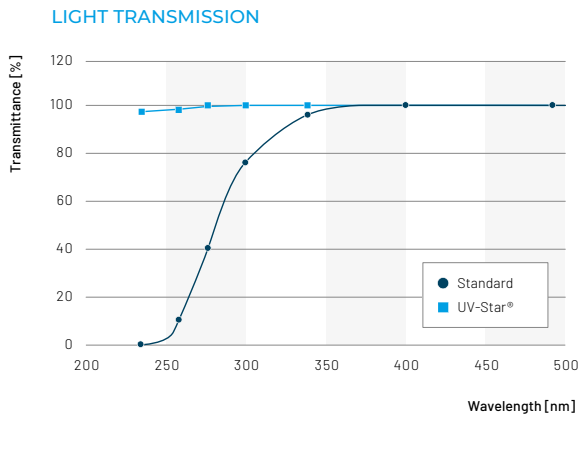

Figure 5: Light transmission of UV-Star® microplates in comparison to standard polystyrene plates.

Color	Bottom	Properties	Application
Black	Solid / µClear®	High-binding/sterile	Immuno assay, growth of microorganisms
		Medium-binding (non-treated)	Biochemical assay
		CELLSTAR®	Cell-based assay
		Advanced TC and CELLCOAT®	Cell-based assay with fastidious cell lines
White	Solid / µClear®	High-binding/sterile	Immuno assay, growth of microorganisms
		Medium-binding (non-treated)	Biochemical assay
		CELLSTAR®	Cell-based assay
		Advanced TC	Cell-based assay with fastidious cell lines

Table 3: Overview of black and white pigmented half area microplates.

tools for measurements in the UV are quartz glass cuvettes with a path length of 1 cm. To achieve this path length within a cuvette, a total sample volume of 1 ml is necessary. The 96 well half area UV-Star® microplate allows significant reduction of sample volume down to 170 µl without impairment of measurement results **(Fig. 6).**

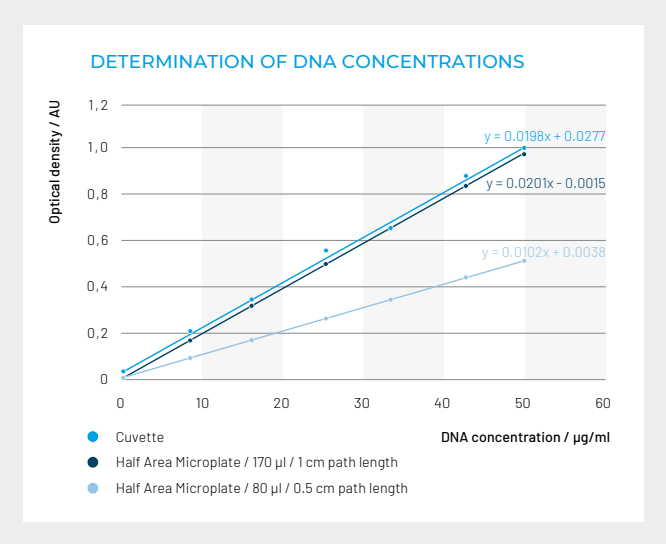

Figure 6: Determination of DNA concentrations in both half area microplates and quartz glass cuvettes with a total volume of 170 µl and 1 ml, respectively, results in comparable OD values.

applications **(Table 3)**. Black or white pigmented microplates help to overcome critical factors frequently linked to such assays like background, autofluorescence or crosstalk. In addition to solid black or white microplates suitable for measurements from the top, pigmented half area plates with a transparent 190 µm thick film bottom are available for detection through well bottoms as well as microscopic analysis **(Fig. 7) [4, 5]**. Manufactured according to a patented processing technique without the use of adhesives or solvents, µClear® plates are available with different surface properties for a variety of applications.

Figure 7: 96 well half area microplates with solid bottom (left) and µClear® film bottom (right)

2.1.3/ FLUORESCENCE AND LUMINESCENCE MEASUREMENTS

Many biochemical and cell-based assays are based on fluorescence or luminescence read outs. For this purpose half area microplates are available in black for fluorescence detection or white for luminescence

2.1.3.1/ FLUORESCENCE-BASED DETERMINATION OF SMALL DNA CONCENTRATION IN BLACK SOLID-BOTTOM 96 WELL MICROPLATES

The determination of small DNA amounts in samples is an important prerequisite for many experiments in

molecular biology. The most commonly used technique for measuring nucleic acid concentration is the determination of absorbance at 260 nm. The absorbance method is relatively insensitive and does not distinguish between DNA and RNA. Nucleic specific fluorescence stains such as is the Quant-iT™ PicoGreen® dsDNA reagent (# P7581, Invitrogen, Carlsbad, CA, USA) circumvents these problems and allows ultrasensitive detection of DNA concentration down to 50 pg. A black half area microplate is the perfect choice for such measurements as it allows cost reduction by conservation of both reagents and by up to 54 %.

A similar signal strength to that of a standard 96 well microplate can be achieved using either less sample material or fluorescent stain within a half area microplate **(Fig. 8)**. Furthermore, a similar quantity of sample material and fluorescence dyes used with a standard volume 96 well microplate leads to significantly higher fluorescence signals **(Fig. 9)** within a 96 well half area microplate due to the higher liquid height in the half area microplate wells.

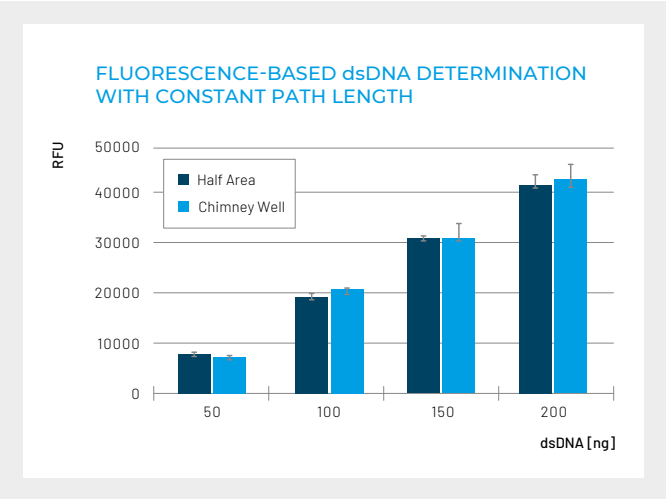

Figure 8: Fluorescence-based determination of dsDNA concentration in 96 well half area and chimney well microplates (Quanti-iT™ DNA Assay Kit, Invitrogen). Measurements resulted in comparable RFU values with a total well volume of 80 µl in half area and 175 µl in chimney well microplates. Measurements were performed according to the supplied protocol.

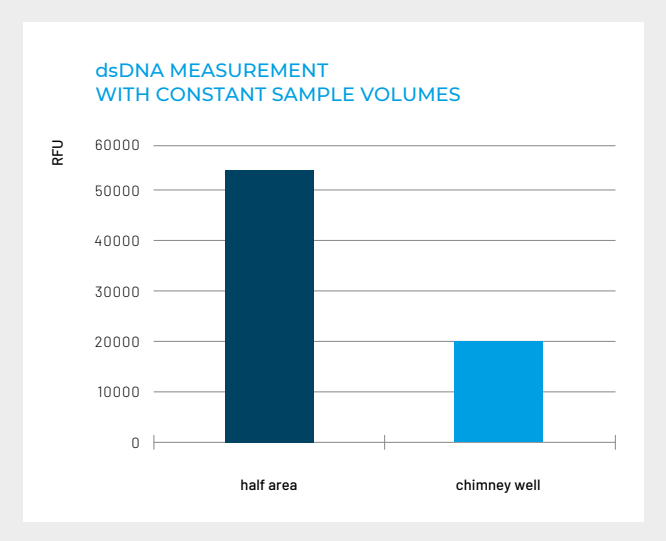

Figure 9: dsDNA determination with propidium iodide. Measurement of the same dsDNA concentration in a total volume of 100 µl resulted in a higher RFU value in half area than in standard volume chimney 96 well microplates.

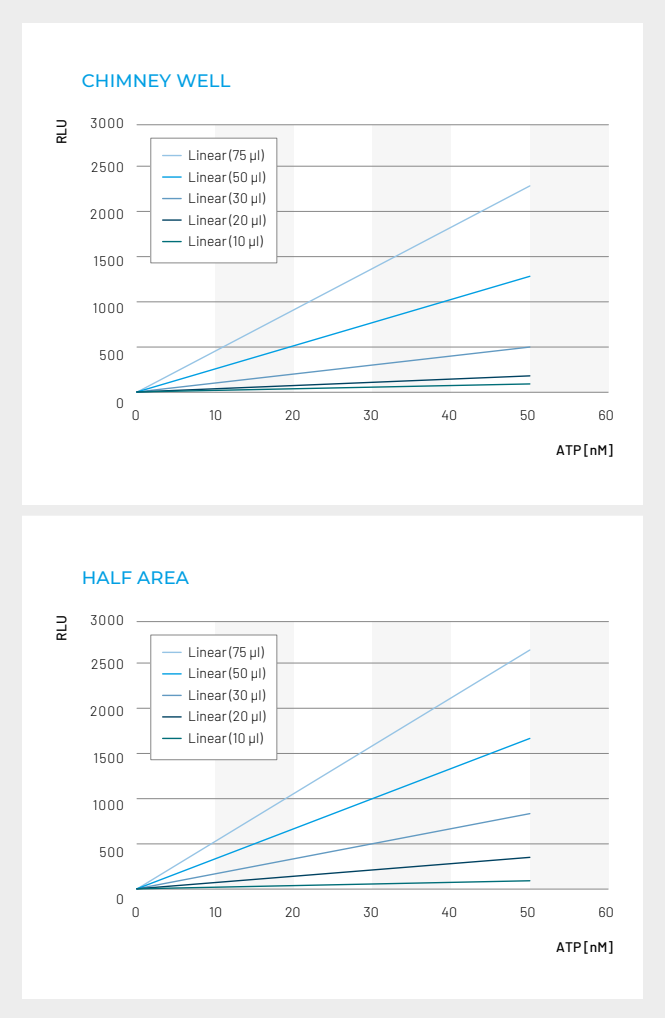

Figure 10: Comparison of total volumes from 10 to 75 µl containing ATP in serial dilutions from 0 to 50 nM in chimney well (top) and half area (bottom) 96 well microplates.

2.1.3.2/ ATP DETERMINATION IN SMALL TOTAL SAMPLE VOLUMES IN 96 WELL HALF AREA MICROPLATES BASED ON LUMINESCENCE MEASUREMENTS

Adenosine triphosphate (ATP), as energy intermediate plays a key role in many biological processes. It serves as the principal immediate donor of energy and is present in all metabolically active cells. Because of its excellent sensitivity which can go down into the attomolar range, bioluminescence is frequently used for the determination of ATP concentration. One method utilised to quantify ATP is a bioluminescence assay based on firefly luciferase activity (# LBR-S010, Biaffin GmbH & Co KG, Germany). In this assay the substrate D-luciferin is oxidised in an ATP-dependent process, generating chemiluminescence.

96 well half area microplates support the sensitivity of the bioluminescence-based ATP measurements, as the half area microplates feature higher signal intensity with smaller sample volumes than those required with standard 96 well microplates **(Fig. 10)**. As with the fluorescence measurements, a similar amount of sample material, enzyme and buffer leads to significantly higher signals in half area versus standard volume 96 well microplates due to the higher liquid height in the half area microplate wells **(Fig. 11)**.

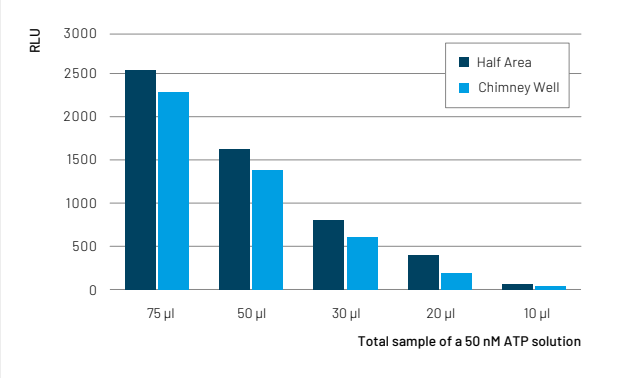

Figure 11: Comparison of Relative Luminscence Units [RLU] values of a 50 nM ATP solution with total volumes of 10 to 75 µl in a half area and standard 96 well microplate. Bioluminescence signal strength is dependent on assay volume. The measurement in half area microplates results in higher gradients and signal strengths than those obtained with the same sample volumes in standard 96 well microplates.

2.2/ CULTIVATION IN HALF AREA MICROPLATES

The reduced well diameter and growth area of half area microplates allow a significant reduction of both media volume and cell number used for initial seeding. Therefore half area microplates are especially useful for cultivations where expensive media supplements are required or the quantity of available cells is limited.

Half area plates are available with different surface properties to support the special needs for cultivation of adherent and suspension cells or organisms such as bacteria, yeast, algae and biofilms **(Table 4)**.

Feature	Application
CELLSTAR® cell culture surface	Adherent cell culture, zebrafish
Advanced TC cell culture surface	Adherent cultivation of fastidious cell lines under restricted growth conditions
Sterile surface	Cultivation of suspension cell culture,bacteria, yeast, algae, biofilms
CELLCOAT® protein coatings	Adherent cultivation of fastidious cell lines and primary cells

Table 4: Half area microplates for cultivation

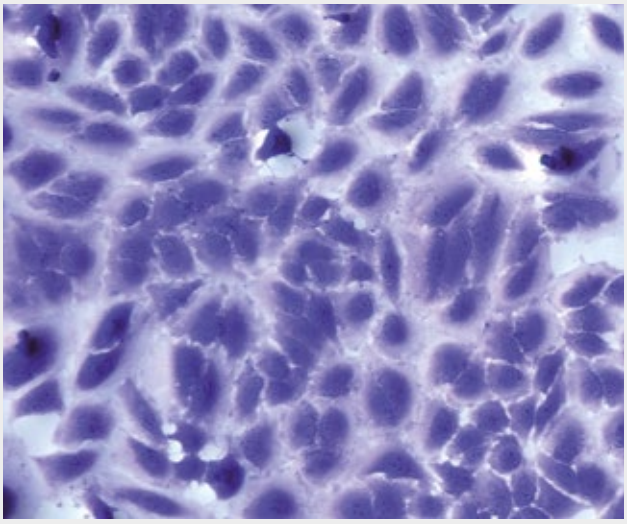

Figure 12: HeLa cells grown in a CELLSTAR® 96 well half area plate stained with crystal violet.

3/ ORDERING INFORMATION

96 Well Half Area Microplates

Well format: 96, Growth area/unit: 15 mm², Well profile: F-bottom, Raw material: PS, Plate design: half area, Working volume (well): 15 µl - 175 µl

Item No.	Bottom	Binding characteristics / Surface treatment	Product colour	Sterile	Lid	Qty. inner / outer
675101	solid	untreated	○ clear			10 / 40
675161	solid	untreated	○ clear	•		10 / 40
675074	solid	high-binding	○ white	•		10 / 40
675075	solid	untreated	○ white			10 / 40
675077	solid	high-binding	● black	•		10 / 40
675076	solid	untreated	● black			10 / 40
675096	µClear®	untreated	● black			10 / 40
675061	solid	high-binding MICROLON 600	○ clear			10 / 40
675001	solid	medium-binding MICROLON 200	○ clear			10 / 40
675180	solid	TC	○ clear	•	•	8 / 32
675083	solid	TC	○ white	•	•	8 / 32
675086	solid	TC	● black	•	•	8 / 32
675090	µClear®	TC	● black	•	•	8 / 32
675986	µClear®	Advanced TC	● black	•	•	8 / 32
675801	µClear®	UV-Star®	○ clear			10 / 40

4/ LITERATURE

i Misselwitz B. et al. (2011) RNAi screen of Salmonella invasion shows role of COPI in membrane targeting of cholesterol and Cdc42. Molecular Systems Biology 7:474.

ii Application Note „UV/VIS Spectroscopy in Microplates" (F073 041), Greiner Bio-One GmbH.

iii Application Note „Insulin ELISA on high binding MICROLON® 600 and CELLSTAR® Microplates" (F073 106), Greiner Bio-One GmbH.

iv Kunze B. et al. (2010) Damage of Streptococcus mutans biofilms by carolacton, a secondary metabolite from the myxobacterium Sorangium cellulosum. BMC Microbiology 10:199.

v Rajkowitsch L. and Schroeder R. (2007) Coupling RNA annealing and strand displacement: a FRET-based microplate reader assay for RNA chaperone activity. BioTechniques 43: 304 – 310.

vi Hoffmann C. et al. (2010) In Macrophages, Cascade-1 Activation by SopE and the Type III Secretion System-1 of S. thyphimurium can proceed in the absence of flagellin. PLoS One 5:12477.

vii Bernstein D. I. et al. (2010) Randomized, double-blind, phase 1 trial of an alphavirus replicon vaccine for cytomegalovirus in CMV seronegative adult volunteers. Vaccine 28: 484 - 493

viii Vogt A. et al. (2009) Automated image-based phenotypic analysis in zebrafish embryos. Dev Dyn 238: 656 – 663

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