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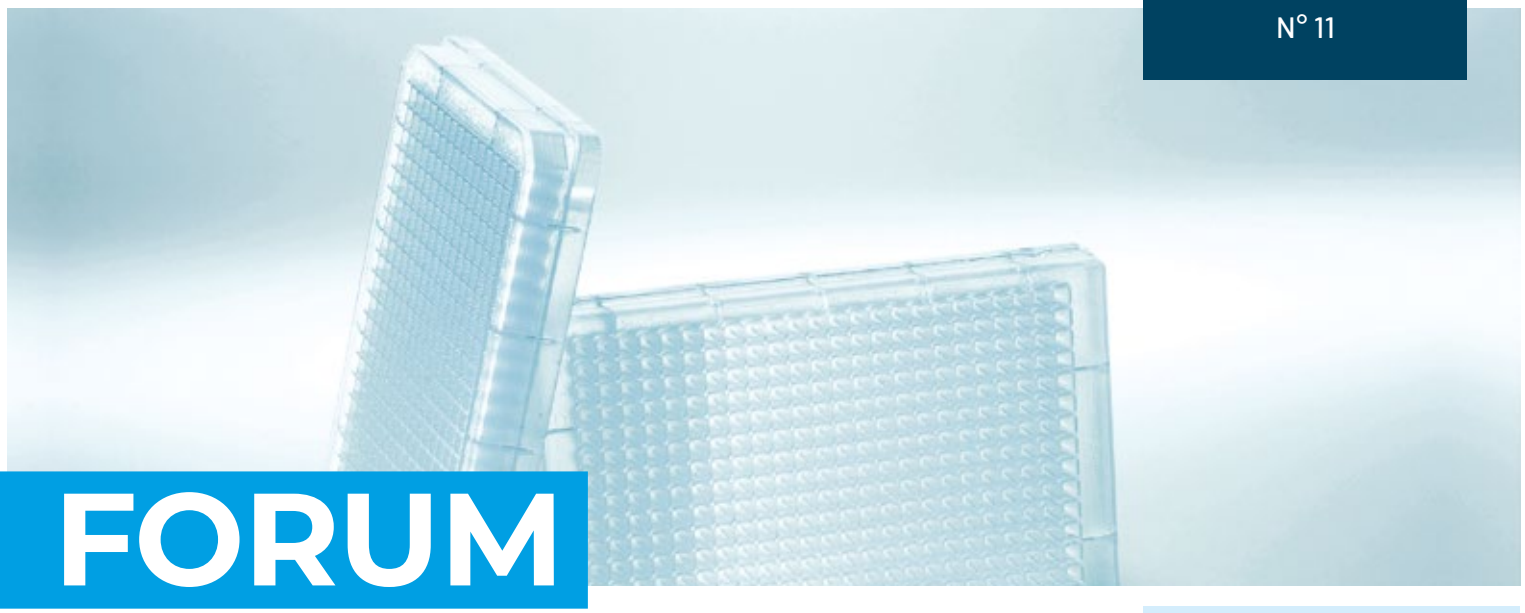

FORUM

TECHNICAL NOTES & APPLICATIONS FOR LABORATORY WORK

CONTENT

A 384 WELL STORAGE PLATE REDUCING COMPOUND CONSUMPTION AND SUPPORTING ASSAY MINIATURISATION

1/ SAMPLE STORAGE IN DRUG DISCOVERY, DIAGNOSTICS AND BASIC RESEARCH

Reliable sample storage and retrieval is a vital function to drug discovery as well as basic research and diagnostics. Coordination and distribution of samples and compounds is the initial step in a cascade of subsequent actions within an experiment.

Errors in sample distribution, many of which are difficult to trace, frequently lead to inaccurate or misleading experimental results. Trouble-shooting the root cause of inaccurate data can be very time-consuming and therefore linked with high costs.

For storage of a relatively small number of samples, storage tubes and box systems with handwritten labels are frequently used.

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In these systems tubes are customarily labelled with indelible ink, however, the small writing area severely limits the amount of information which can be contained on the tube, and the stability of the label is not guaranteed **(Fig. 1)**.

Because of these disadvantages, use of hand-labelled storage systems often results in non-identifiable samples,

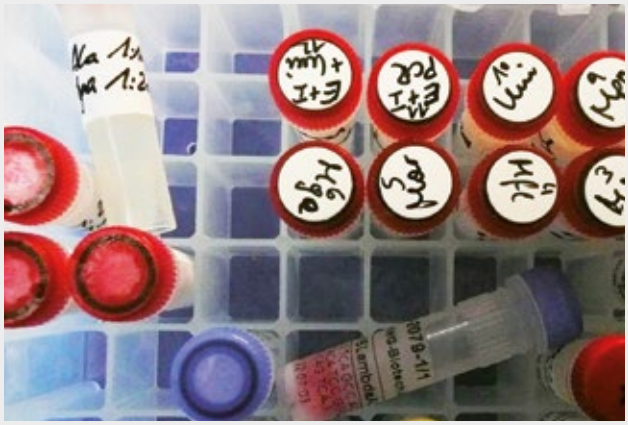


Figure 1: Example for storage of samples in basic research. The samples are stored in labelled polypropylene tubes in paperboard boxes at -20 °C or -80 °C. The amount of information written on the tube is limited. An organised storage structure is difficult to achieve.

confusion and loss. For this reason, systematic storage systems have become increasingly popular, even in basic research laboratories.

An intelligent sample management system is the use of cryogenic conservation tubes labelled with laser-printed 2D barcodes. This system allows tube storage in boxes **(Fig. 2)**, but with significant improvement in the amount of printed information that can be contained on the tubes.

Figure 2: Cryo.s tubes with Datamatrix for cryogenic sample storage and secure and efficient sample identification.

Printed 2D barcodes are highly resistant against many solvents used in the laboratory and do not smudge or blur. The integrity of the code will remain unchanged throughout the life span of the tube. Together with simple personal computer based data management systems and commercially available 2D barcode readers, 2D barcoded tubes provide a safe and reliable storage system for basic research, diagnostics and smaller drug discovery groups (further information: Greiner Bio-One Forum No. 10, Datamatrix Coding).

1.1/ MICROPLATES FOR AUTOMATED SAMPLE STORAGE

With the increase of throughput, a sophisticated solution with automation is mandatory. Microplates are frequently the tool of choice for automated sample storage **(Fig. 3)**.

Due to the efforts of the Microplate Standards Development Committee of the Society for Biomolecular Sciences, primary microplate dimensions (e.g., length, width and height) and tolerances are standardised according to the American National Standards Institute (ANSI), which facilitates automation.

Figure 3: Barcoded storage microplate in a high-throughput screening environment.

Picture courtesy of Boehringer Ingelheim (www.boehringer-ingelheim.com)

1.2/ CHEMICAL CHARACTERISTICS OF POLYPROPYLENE

Most storage microplates are manufactured of polypropylene **(Fig. 4)**.

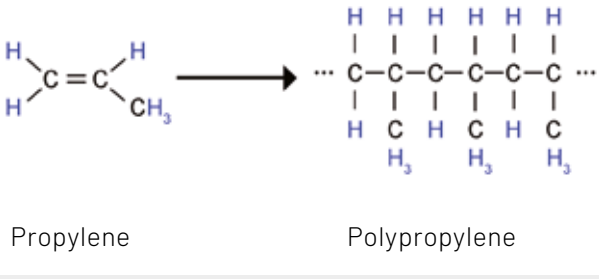


Figure 4: Chemical structure of polyproyplene and its monomer propylene.

Polypropylene (PP) has low biomolecular binding characteristics **(Fig. 6)**, high temperature tolerance and high resistance against many solvents such as DMSO **(Table 1)**. Polypropylene can also easily be heat-sealed, a major request in high-throughput screening where heat sealing is the most common way to close a microplate.

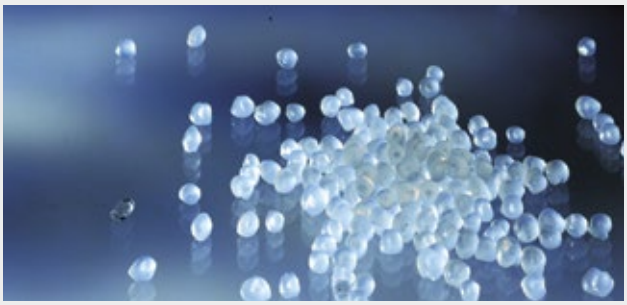


Figure 5: Polyproyplene resin

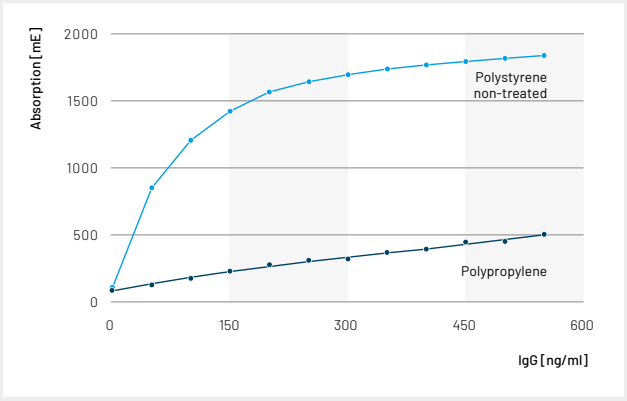


Figure 6: Binding of human IgG to polystyrene and polypropylene.

	PP 20 °C	PP 50 °C
Acetone	1	3
Acetonitrile	3	4
Chloroform (CHCl3)	3	4
Cyclohexanol	1	3
Detergents	1	1
Dimethylsulfoxide (DMSO)	1	1
Ethanol 96%	1	1
Hexanol	1	-
Isobutanol	1	1
Isopropanol	1	1
Methanol	1	1
Phenol (100 %)	1	1
Sulfuric Acid 60%	1	3
Tetrachlormethane	4	4

Table 1: Chemical resistance of polypropylene.
1 = resistant, 2 = limited resistant, 3 = moderate resistant, 4 = no resistance

This is a general guide only. As many factors can affect the chemical resistance of a given product, its suitability for a specific application should be tested. For more detailed information about chemical resistance of different raw materials please visit our website: www.gbo.com

1.3/ MICROPLATES FOR COMPOUND MANAGEMENT

A large variety of 96 well and 384 well storage microplates are commercially available. 96 well microplates are widely used in research or diagnostics, whereas drug discovery is more focused on miniaturisation, and 384 well or even 1536 well microplates are frequently applied for the storage of compound solutions. Microplates with different well designs are available for these formats **(Fig. 7)**. For many years microplates with conical V-shaped wells **(Fig. 8)** were preferred in high-throughput screening, as it was generally assumed this design enabled better pipetting with all sample collected in a central location at the well bottom. With increasing popularity of direct compound transfer, the classical F- and V- bottom well designs **(Fig. 8)** do not fulfill all requirements of new compound management technologies. It is especially difficult to precisely position pin tools or pipette tips for reliable access of low sample volumes, resulting in loss of valuable sample material.

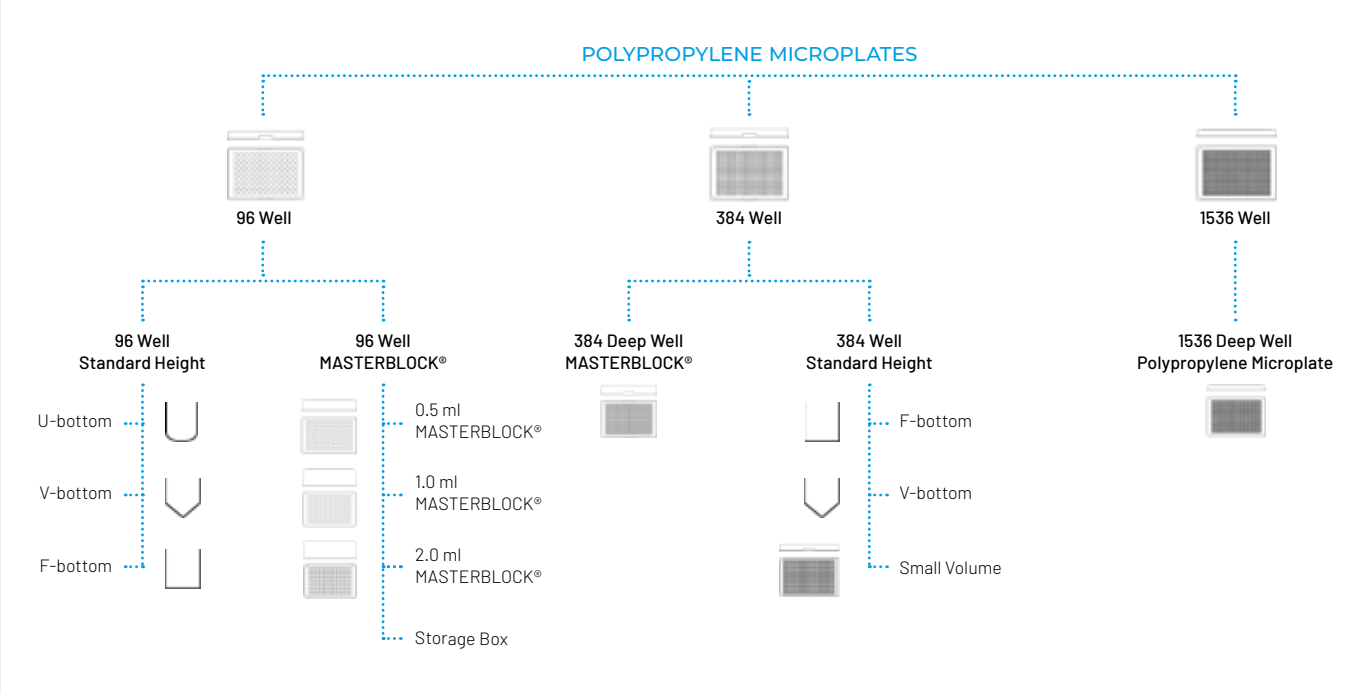
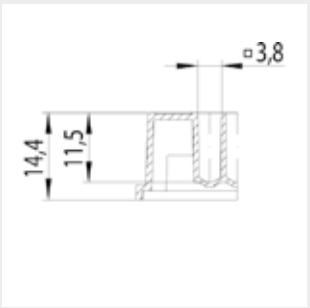
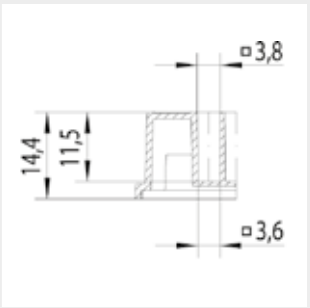


Figure 7: Selection of available polypropylene microplates.

Figure 8 : V- and F-bottom well design:
The V stands for a conically tapered well whereas F stands for a flat bottom well.



Well profile: 384 well V-bottom, polypropylene
Total volume: 130 µl
Working volume: 13-120 µl



Well profile: 384 well F-bottom, polypropylene
Total volume: 152 µl
Working volume: 15-145 µl

1.4/ CONCEPT OF DIRECT COMPOUND TRANSFER

In direct compound transfer **(Fig. 9)** very low volumes of compound solutions are transferred from a source plate directly into an assay plate without any intermediate dilution steps. The compounds are then diluted to the desired final concentration by adding or pre-dispensing assay buffer and reagents.

Direct compound transfer **(Fig. 9)** saves labor and costs with elimination of redundant intermediate dilution steps. Furthermore, minimising dilution steps additionally reduces the risk of compound precipitation. Because non-diluted compounds are often in scarce supply, a low dead volume of the storage microplate is highly desirable to achieve complete sample retrieval with minimal waste. Direct compound transfer can be performed either with pin tools **(Fig. 10)** or with acoustic liquid handling systems. Acoustic liquid handling enables the transfer of very small amounts of compounds down to 1 nl, but requires expensive technical equipment, whereas pin tools are much easier and less costly to implement in existing high-throughput screening environments and allow the liquid transfer of approximately 50 nl.
Aditionally, acoustic liquid handling systems require

Figure 10: Low Dead Volume Liquid Handling / Capillary Head for CyBi®-Well vario.

- Simple and robust
- Aspiration by capillary effect
- Calibrated capillaries with fixed volumes
- Dispensing by pressure impulse / air pressure
- Simultaneous transfer of 96 or 384 samples
- Low dead Volume (< 1 µl)

Picture courtesy of Cybio AG (www.cybio-ag.com)

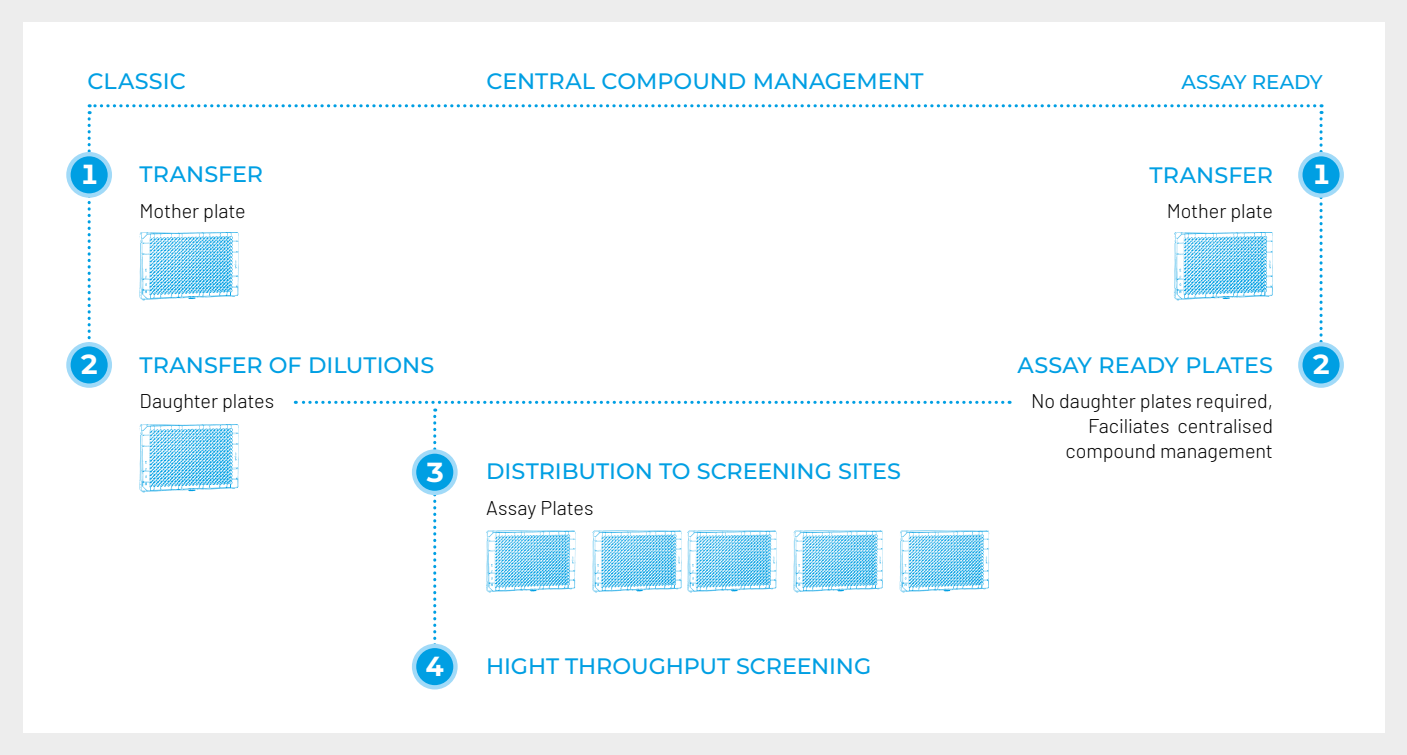


Figure 9: Example of a direct compound transfer approach in comparison to a classic approach with diluted compounds.

specialised microplates, while pin tools can be employed with all kind of existing storage microplates. However, a disadvantage of pin tools is that they may be sensitive to an uneven liquid distribution in standard V- and F-bottom microplates, leading to loss of valuable compounds.

To overcome the drawback of conventional polypropylene storage microplates a new well geometry **(Fig. 11)** was developed in cooperation with the Compound Management and HTS groups of Boehringer Ingelheim (Biberach, Germany). The resulting 384 Deep Well Small Volume microplate was designed to allow perfected positioning for small sample volumes with pin tools or pipette tips and facilitate direct transfer from storage to assay plates without need for expensive devices.

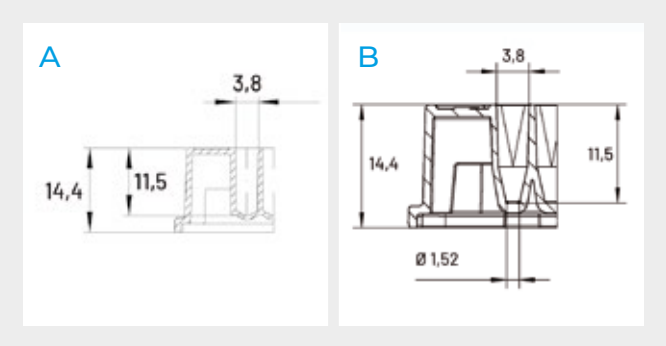


Figure 11: Well geometry of a standard V-bottom (A) well and well geometry of the 384 Deep Well Small Volume microplate (B).

2/ DEVELOPMENT OF A STORAGE PLATE SUPPORTING ASSAY MINIATURISATION IN HIGH-THROUGHOUT SCREENING

2.1/ DETERMINING THE BEST WELL GEOMETRY

During initial development a technical specification for the well geometry of the microplate was postulated:

- **The positioning of small sample volumes must be precisely focused in the well cone so that the sample can be easily transferred by pin tools or pipette tips.**
- **The plate must follow the most important ANSI recommendations (length, width, height, curvature) to be compatible with robotic liquid handling systems and common microplate handling processes (e.g. heat sealing, piercing, stacking).**
- **The wells must have a maximal volume of approximately 100 µl to enable pre-dilution of samples.**

To determine the optimal well cone geometry a hybrid prototype microplate with three different cone geometries was designed **(Fig. 12)**.

- **Design 1: conical well with a round bottom**
- **Design 2: conical well with a flat bottom**
- **Design 3: blunt cone with a round bottom**

Figure 12: Different well geometries.
A) Design 1 – Conical well with round bottom
B) Design 2 – Conical well with flat bottom
C) Design 3 – Blunt cone with a round bottom.

The resulting hybrid test plate was evaluated in practical tests. The wells were tested with 50 nl and 100 nl pin tools by the HTS group of Boehringer Ingelheim in Biberach according to the following procedure:

- **Filling of the plate with 1 µl and 2 µl DMSO Orange G solution, respectively (Flexdrop, Perkin Elmer, Waltham-Massachusetts)**
- **Visual control and centrifugation**
- **Transfer with 50 nl and 100 nl pin tools into an assay plate (20/40 µl aqueous buffer solution, V&P Scientific)**
- **Validation of the transfer precision by absorption measurement (TECAN Ultra)**

The results of the first tests were:

- **Design 1 showed splashing with some pipetting devices.**
- **Pin tools can be damaged with design 3 if they touch the well wall.**
- **Design 2 showed the best performance in liquid transfer (Table 2).**

			Design 1	Design 2	Design 3
50 nl pin tool	from 1 µl into 20 µl	CV [%]	6.410	2.982	4.480
		Min [OD]	0.384	0.528	0.487
		Max [OD]	0.618	0.616	0.601
		Vol. [nl]	53	53	51
	from 2 µl into 20 µl	CV [%]	4.533	2.849	5.209
		Min [OD]	0.523	0.577	0.508
		Max [OD]	0.675	0.660	0.679
		Vol. [nl]	58	58	58
100 nl pin tool	from 2 µl into 40 µl	CV [%]	3.075	3.895	3.310
		Min [OD]	1.088	1.095	1.025
		Max [OD]	1.246	1.253	1.211
		Vol. [nl]	106	108	101
	from 1 µl into 40 µl	CV [%]	5.501	5.726	4.789
		Min [OD]	0.952	0.905	0.787
		Max [OD]	1.152	1.148	1.098
		Vol. [nl]	95	96	90

Table 2: Pin tool liquid transfer from different wells.
Data courtesy of Boehringer Ingelheim (www.boehringer-ingelheim.com)

- **The flat well bottom of design 2 was easily accessible in contrast to the standard V-bottom design (Fig. 15).**
- **The flat bottom slightly reduced the liquid height for volumes below 1 µl but concentrated the sample in the well cone (Fig. 13).**

Further tests with colored DMSO solution demonstrated that the remaining liquid, e.g. compounds in DMSO,

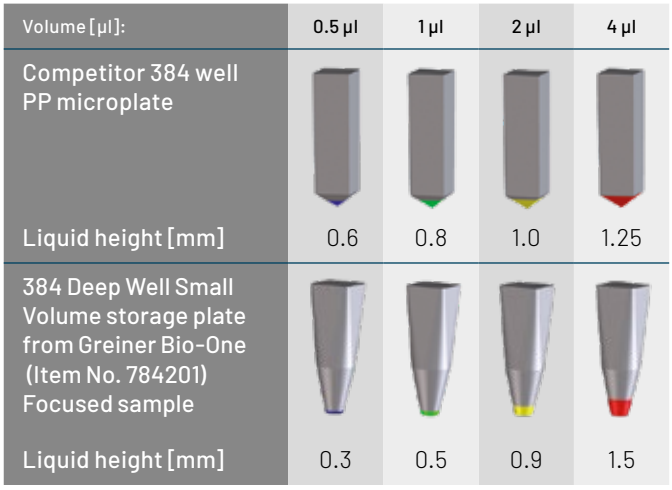


Figure 13: Liquid height depending to well volume.

aggregate in the well bottoms of design 2, whereas the liquid within standard V-bottom wells tended to spread out **(Fig. 15)**. Furthermore the liquid height level increased at a faster rate in design 2 in comparison to the classical V bottom well **(Fig. 13)**. With the combination of a clearly focused access for pin tools **(Fig. 15)**, sufficient tolerances to avoid crashes and limited space to avoid liquid distribution, well design 2 allowed a precise direct compound transfer for the postulated specification criteria. In summary, design 2 exhibited the best performance and was therefore selected as the basis for the well design.

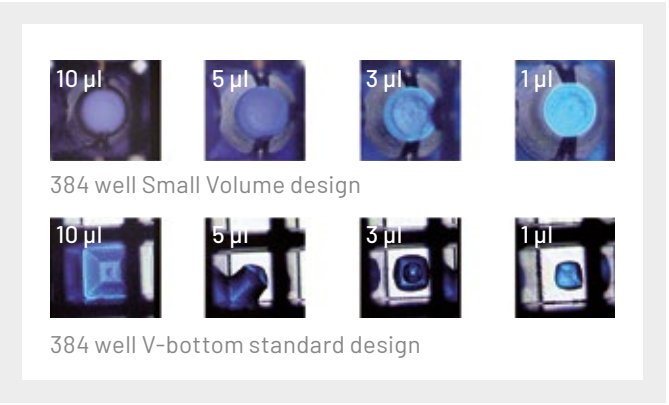


Figure 14: Location of liquid at the bottom of the well in comparison to a standard V-bottom well.

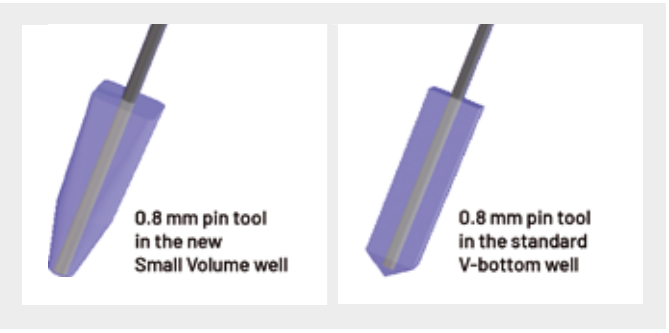


Figure 15: Positioning of pin tools.

2.2/ PERFORMANCE OF THE 384 DEEP WELL SMALL VOLUME PLATE FOR PRE-DILUTIONS

Despite advantages of direct compound transfer, many assays still require a classic approach with pre-diluted compound solutions. Although the preparation of diluted compounds facilitates homogenous mixing of compounds with assay buffer, direct compound transfers may not be

the best method for cell based assays. This especially because adherent cells can react sensitively when placed in direct contact with undiluted, highly concentrated compounds dissolved in 100 % DMSO. Furthermore, studies have not yet completely examined the behavior of compounds when stored at very low volumes for long time periods in assay microplates. Open questions remain as to evaporation, compound solubility, and binding of compounds to the microplate surface.

Another major requirement of the well design was a maximal volume of approximately 100 µl to enable predilution of samples. To achieve the specified total volume the well cone was combined with a square well geometry at the top **(Fig. 16 A)**, resulting in a final working volume of 1- 90 µl and a maximal volume of 107 µl **(Fig. 16 B)**.

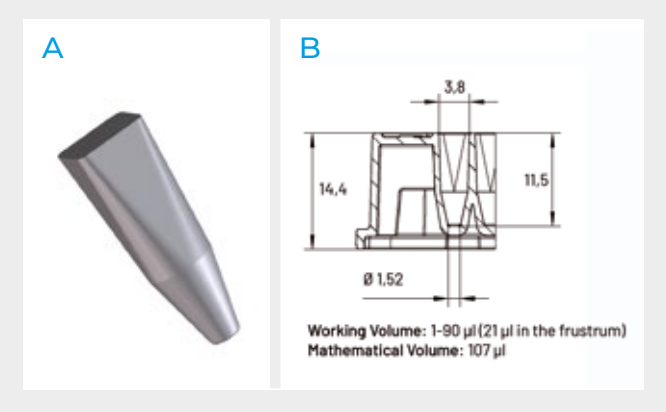


Figure 16: Shape of the well design.

2.3/ USE OF THE 384 DEEP WELL SMALL VOLUME PLATE IN EXISTING HIGH-THROUGHPUT SCREENING SYSTEMS

The outer dimensions and the height of the microplate are following the ANSI recommendations **(Fig 17 / ANSI-SBS1-2004, ANSI-SBS2-2004)**. The depth of the well is similar to well depth in a standard Greiner Bio-One F-bottom or V-bottom microplate, thereby avoiding a time consuming adaption in the automation process. Relevant product information (technical drawing, data sheet) is available on our website (www.gbo.com/bioscience) in our online shop.

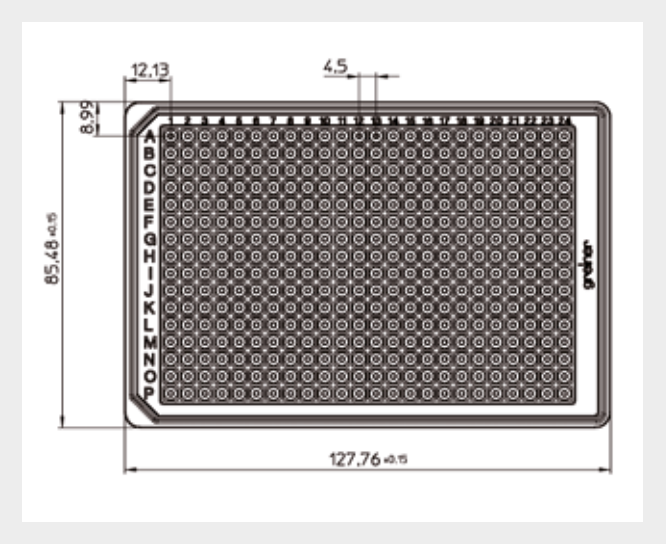


Figure 17: Microplate dimension of the 384 Deep Well Small Volume microplate.

3/ HEAT SEALING

Another major requirement for the microplate design was compatibility with heat sealing. Heat sealing is the most widespread applied technology for closing compound storage microplates. Heat seals are temperature and DMSO resistant and chemically inert. Most heat sealing devices available on the market work in a similar manner. A polypropylene coated film, either a transparent plastic film or an aluminum foil, is "ironed" onto the microplate surface.

To enable a tight heat seal the 384 Deep Well Small Volume microplate provides a sufficiently pronounced rim of 0.5 mm and is characterised by tight tolerances to improve its sealing properties. No matter which device is used, for a tight and non-destructive sealing the right settings must be evaluated carefully before the onset of routine work. Each microplate design needs special adjustments of the heat sealing parameters (temperature, time and pressure) due to the influence of wall thickness, well design and raw material on heat sealing properties.

Device	Temperature	Time	Pressure[(x)] Adapters	Result
Remp Portrait Heat Sealer	168 °C	2 sec	Adapter	Plate completely sealed, no heat distortion and warpage minimised. Sealing Device should be adjusted to the plates height of 14.4 mm in order to avoid high sealing pressure
Velocity11 PlateLoc®	164 °C	2 sec	81 PSI	Plate completely sealed, no heat distortion and warpage minimised. Several adapters supplied from Velocity can improve the sealing results
Velocity11 PlateLoc®	170 °C	1 sec	81 PSI	Alternative PlateLoc® setting: Plate completely sealed, no heat distortion and warpage minimised. Several adapters supplied from Velocity can improve the sealing results
ABGene ALPS 300	172 °C	1 sec	Adapter	Plate completely sealed / No heat distortion / Warpage minimised

Table 3: General guideline for heat sealing settings in a Remp (Remp AG, Oberdiessbach, Switzerland), Velocity11 PlateLoc® (Velocity11 Automation Solutions, Santa Clara, CA USA) and ABGene ALP 300 (ABGene, Epsom, UK) for the 384 Deep Well Small Volume (Item No. 784201).

3.1/ HEAT SEALING PARAMETERS AND THEIR INFLUENCE ON SEALING PROPERTIES

1) TEMPERATURE / TIME

It is recommended to limit the sealing time as much as possible. Higher temperatures with a shorter sealing time will generally yield a better result (less warpage) than a longer sealing time with lower sealing temperatures.

2) INFLUENCE OF POLYPROPYLENE

The polypropylene resin used for microplate manufacture can influence its sealing properties. Microplates manufactured of polypropylene with a high melting point require stricter settings (higher temperature, longer sealing time) than microplates manufactured from lower melting point polypropylene.

Storage microplates from Greiner Bio-One are generally manufactured from the same type of raw material, which facilitates the transfer of existing heat sealing settings.

3) PLATE HEIGHT

The adjustment of the heat sealing device to the individual microplate height is essential for a reliable sealing process. Inaccurate height settings can lead to a distorted microplate surface or incomplete heat sealing **(Fig. 18)**. All Greiner Bio-One 384 well standard volume polypropylene microplates have a height of 14.4 mm (ANSI/SBS2-2004 Height Dimensions) which facilitates the setup of heat sealing processes.

4) ADAPTERS

Most suppliers of heat sealing systems offer adapters that support the centre of the microplate. Adapters are advantageous to distribute pressure, thereby reducing the potential for warpage and heat distortion of the sealed microplate.

5) PRESSURE

Pressure is not generally defined as an adjustable parameter. However, pressure may have an influence on the

sealing of the microplates as well. For detailed technical support please contact the supplier of the heat sealing device.

6) MULTIPLE HEAT SEALING

Multiple heat sealing offers an advantage in that microplates can be opened and closed several times for compound sampling, however it should be noted that each heat sealing step translates to a stress for both the microplate and the material it is manufactured of. Therefore multiple heat sealings are not recommended, as multiple heat-sealed microplates often demonstrate warpage and deformation. The resulting microplate geometry, tolerances and dimensions will not fulfill the tight specifications necessary for hassle-free high-throughput screening.

As an alternative to multiple heat sealings, remaining compounds should be transferred into separate microplates or the concept of compound storage based on sealing with a lid should be selected (M. Pfeiffer, G. Scheel, Journal of Biomolecular Screening 2009, 14 492-498) .

Table 3 provides a general overview for heat sealing the 384 Deep Well Small Volume microplate (Item No. 784201)*.

* This information is a general guide only. As many factors can affect the sealing setting, suitability for a specific application should be tested.

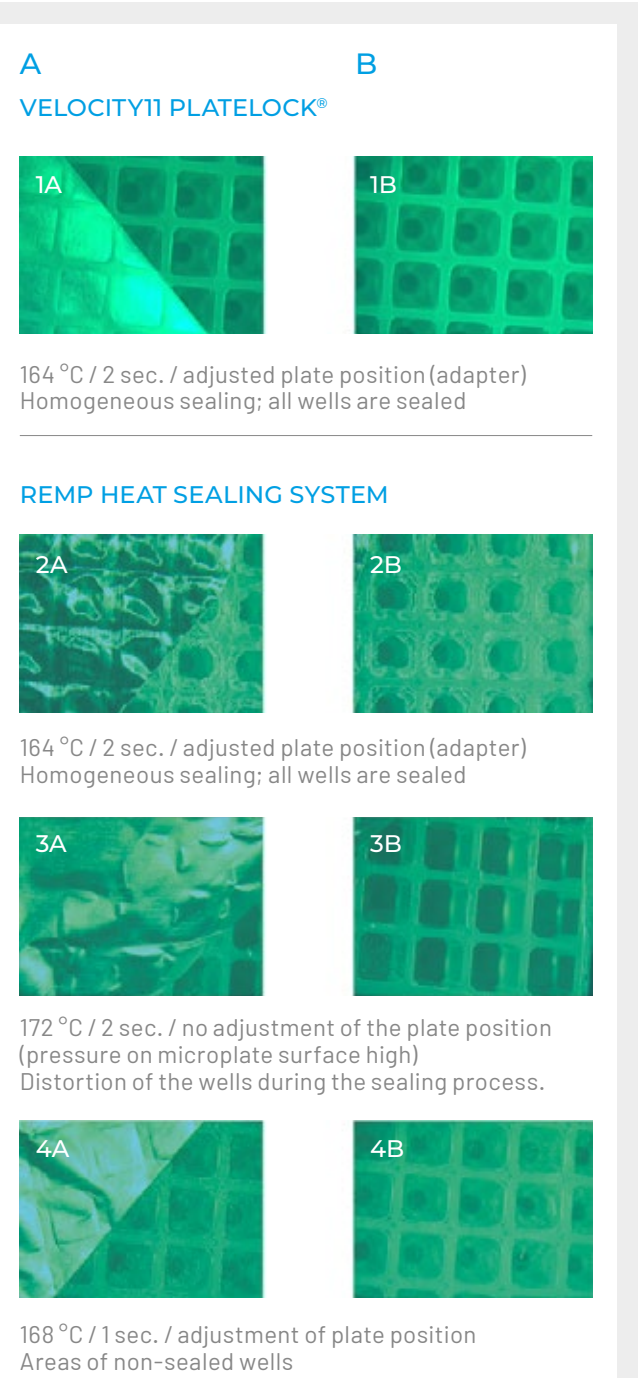


Figure 18: 384 Deep Well Small Volume microplate (Item No. 784201) sealed with different heat sealing devices under different sealing settings. The pictures in column A show the heat sealed microplate surface and the accompanying heat sealing film. The pictures in row B show the surface of heat sealed microplates.

4/ ORDERING INFORMATION

96 Well Polypropylene Microplates

Well format: 96, Bottom: solid, Raw material: PP, Surface treatement: untreated, Lid: no

Item No.	Well profile	Product colour	Working volume (well)	Sterile	Qty. inner/outer
650201	U-bottom/Chimney well	○ natural	50 µl - 300 µl		10/100
650261	U-bottom/Chimney well	○ natural	50 µl - 300 µl	+	10/100
650209	U-bottom/Chimney well	● black	50 µl - 300 µl		10/100
655201	F-bottom/Chimney well	○ natural	25 µl - 370 µl		10/100
655261	F-bottom/Chimney well	○ natural	25 µl - 370 µl	+	10/100
655209	F-bottom/Chimney well	● black	25 µl - 370 µl		10/100
651201	V-bottom/Chimney well	○ natural	50 µl - 335 µl		10/100
651261	V-bottom/Chimney well	○ natural	50 µl - 335 µl	+	10/100
651209	V-bottom/Chimney well	● black	50 µl - 335 µl		10/100

Plates in other colours are available on request.

96 Well MASTERBLOCK®

Well format: 96, Bottom: solid, Raw material: PP

Item No.	Well profile	Product colour	Total volume (well)	Lid	Sterile	Qty. inner/outer
786201	V-bottom	○ natural	0.5 ml	CapMat 381070, 381061		8/80
786261	V-bottom	○ natural	0.5 ml	CapMat 381070, 381061	+	1/80
780201	U-bottom	○ natural	1 ml	CapMat 381070, 381061		1/50
780261	U-bottom	○ natural	1 ml	CapMat 381070, 381061	+	1/50
780215	U-bottom	○ natural	1 ml	CapMat 381070, 381061		5/50
780270	V-bottom	○ natural	2 ml	CapMat 381080, 381081		1/50
780271	V-bottom	○ natural	2 ml	CapMat 381080, 381081	+	1/50
780285	V-bottom	○ natural	2 ml	CapMat 381080, 381081		5/50

96 Well Storage Box

Well format: 96, Raw material: PC

Item No.	Sterile	Qty. inner/outer
975502		1/120
975561	+	1/50
975570		1/50

4/ ORDERING INFORMATION

384 Well Polypropylene Microplates

Well format: 384, Bottom: solid, Raw material: PP, Surface treatment: untreated, Lid: no

Item No.	Well profile	Product colour	Plate Geometry	Working volume (well)	Qty. inner/outer
781201	F-bottom	○ natural		15 µl - 145 µl	10/100
784201	V-bottom	○ natural	Deep Well Small Volume	1 µl - 90 µl	10/100
781201-906	F-bottom	○ natural		15 µl - 145 µl	10/100
781209	F-bottom	● black		15 µl - 145 µl	10/100
781280	V-bottom	○ natural		13 µl - 120 µl	10/100

384 Deep Well MASTERBLOCK®

Well format: 384, Well profile: V-bottom, Bottom: solid, Raw material: PP, Plate geometry: Deep Well, Lid: no

Item No.	Product colour	Sterile	Qty. inner/outer
781270	○ natural		6/60
781271	○ natural	+	6/60

1536 Deep Well Microplates

Well format: 1536, Well profile: V-bottom, Bottom: solid, Raw material: PP, Plate geometry: Deep Well, Working volume: 3 µl - 15 µl, Lid: no

Item No.	Product colour	Sterile	Qty. inner/outer
782261	○ natural	+	15/60
782270	○ natural		15/60

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