Applications

Note 184

Technical Report

Eppendorf Deepwell Plate: Determination of residual volumes during use with the automated pipetting system Eppendorf ep*Motion*®

Natascha Weiß, Rafal Grzeskowiak, Martin Armbrecht, Philip Müller
Eppendorf AG, Hamburg, Germany

Abstract

Challenging demands are placed on consumables used in automated applications, especially when focusing on avoiding sample loss or providing reproducible experimental conditions. In this Technical Report, we have compared deepwell plates in 96 well as well as 384 well format from different manufacturers, with a focus on residual volume following sample recovery using the pipetting system ep*Motion*®. Superior quality in both plate design and manufacturing of the Eppendorf Deepwell Plates is demonstrated by minimal residual volume of solution remaining in the well. Furthermore, the amount of liquid is nearly identical in each well.

Introduction

Whenever valuable samples or expensive reagents are being used, minimization of sample loss becomes important. One contributing factor is the quantitative volume recovery from tubes and plates. Deepwell plates, and especially deepwell plates of the 384 well format, are almost exclusively used in automated settings. While manual pipetting allows controlled and nearly complete sample aspiration, for technical reasons this is not possible to the same extent during automated applications: A minimum safety distance between the tips of the pipetting system and the well bottom is necessary in order to prevent the tip from coming into contact with the well surface.
The exact distance required is a function of geometry and the production tolerance of the wells and tips, as well as the tolerance of the cones of the instrument's dispensing tools.
Since all these factors play into the theoretical residual volume remaining inside the wells, a short safety distance is advantageous. Therefore, precise movements of the pipetting system, as well as minimal production tolerance of both tips and plates, are necessary prerequisites. Further, the uniform level of all plates plays a critical role; i.e. all well bottoms must be in the exact same plane.

The Eppendorf Deepwell Plates are distinguished by their functional design, including conformity to all applicable SBS standards* and excellent production quality, a combination which results in plates that are highly homogenous and robust. Hence these plates are extremely stable during mixing and centrifugation (g-safe®) [1]. The colored frame featuring high contrast laser-engraving, the OptiTrack® matrix, makes the alpha numerical labeling easy-to-read. The RecoverMax® well design featuring rounded edges and conical bottoms enables residual liquids to flow effectively towards the center of the bottom, where they can be removed with ease [2].
For the purpose of this technical report, the automatic pipetting system Eppendorf ep*Motion* 5070 was used to remove a photometrically detectable solution from deepwell plates by Eppendorf, as well as by other manufacturers, as completely as possible. In order to compare the different plates, the liquid remaining inside each individual well was quantified using a photometer.

* ANSI/SBS 1-2004: Microplates – Footprint Dimensions
ANSI/SBS 2-2004: Microplates – Height Dimensions
ANSI/SBS 3-2004: Microplates – Bottom Outside Flange Dimensions
ANSI/SBS 4-2004: Microplates – Well Positions

Materials and Methods

For each experiment two plates each of the Eppendorf Deepwell Plate 96/2000 µl and 384/200 µl as well as two plates of the corresponding formats by other manufacturers were used. A working solution of 5 mM ABTS (2,2'-azino-bis (3-ethylbenzthiazolin-6-sulfonic acid)) (Roche) was used.

All pipetting steps were performed in the Eppendorf ep*Motion*, set to pipetting mode, using identical tips. The safety distance between tip and well bottom was minimized as described in the Eppendorf Userguide 005 [3]. The volumes of ABTS solution, outlined in table 1, were pipetted into each well, and the exact same volume was subsequently removed during the next step. Water was added to the residual volume remaining inside each well (table 1) and mixed by pipetting up and down. 50 µl each were transferred from each well into an assay plate (Nunc PS 96 well F-bottom plate (#269620)). In order to determine the concentration of ABTS the plate was measured at 340 nm in a Flash-Scan plate reader (Analytik-Jena, Germany).

The residual liquid remaining in each well was quantified by comparison to a standard curve.

Table 1: Pipetting pattern for ep*Motion*

DWP	Pipette tip	Addition and removal* of ABTS solution	Addition of water	Transfer into assay plate
2000 µl	300 µl	200 µl	200 µl	50 µl
200 µl	50 µl	50 µl	100 µl	50 µl

*In order to remove the exact same amount of liquid, the ep*Motion* needs to be instructed that more liquid is present inside the wells than there actually is, as the residual volumes remaining in the wells due to the safety distance is already taken into account by the instrument.

The protocol did not take into consideration the fact that the total volume (residual volume + added water) will increase with increasing residual volume. Since these residual volumes are small in relation to the water, this error is negligible.

Results and Discussion

Data obtained from both plate formats demonstrate that the residual volumes remaining inside the deepwell plates by Eppendorf are smaller than the liquid remaining in the tested plates of other manufacturers (Fig. 1 a+b, 2 a–e, 3 a–d). On average, less than 2 µl remain in each well of an Eppendorf Deepwell Plate 96/2000 µl and less than 0.15 µl remain in each well of the 384/200 µl plate. These values are higher in competitors' plates (Fig. 1). The color graphics (Fig. 2+3) clearly show that in addition to retaining a very small volume, these small volumes remaining in Eppendorf Deepwell Plates are highly homogenous. Other manufacturers' plates, especially those from manufacturer G, display larger variations. These variations may be a consequence of the plate material and production process, or they may be due to well geometry.

Fig. 1a: Residual volumes deepwell plate 96 wells – 2000 µl

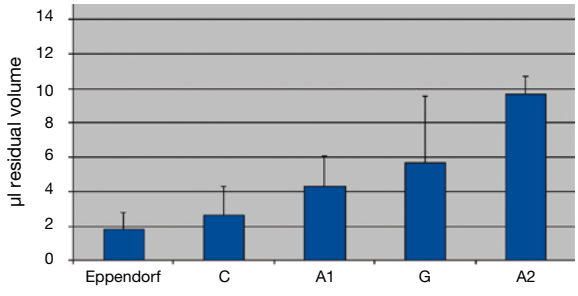
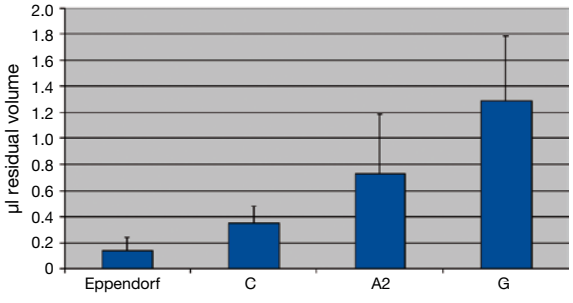
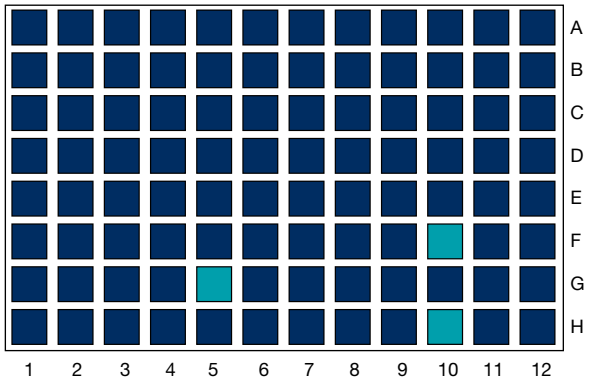


Fig. 1b: Residual volumes deepwell plates 384 wells – 200 µl

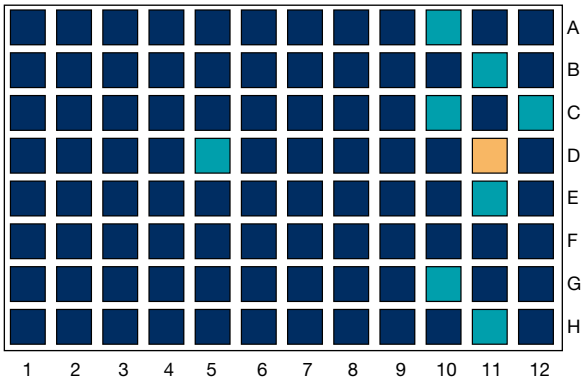


Figures 1 a and b: Averages and standard deviations of residual volumes in all deepwell plates tested.
(a) 96 wells, 2000 µl (n=96); (b) 384 wells, 200 µl (n=384).

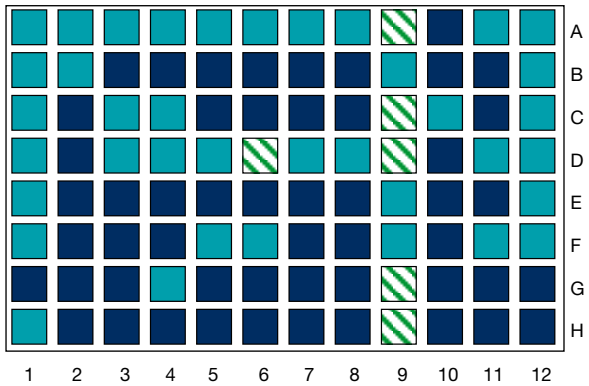
2a Eppendorf Deepwell Plate 96/2000 µl



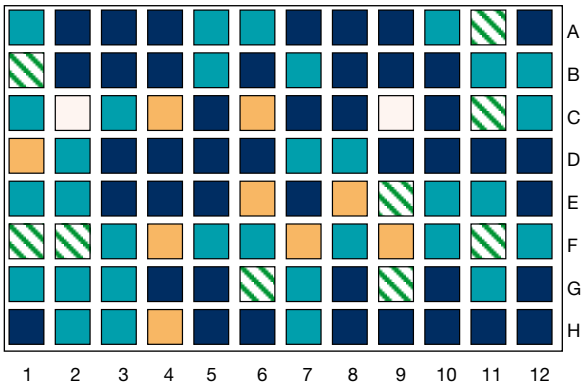
2b Competitor C – 96 wells, 2000 µl



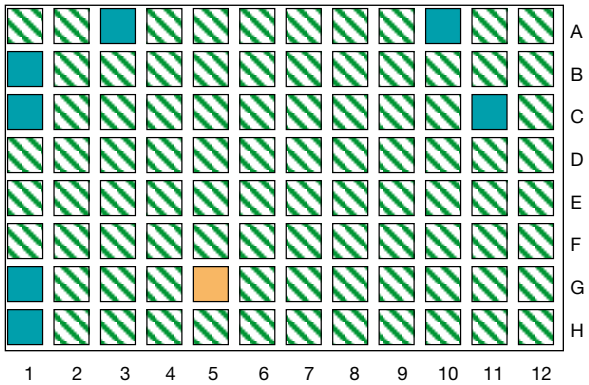
2c Competitor A1 – 96 wells, 2000 µl



2d Competitor G – 96 wells, 2000 µl

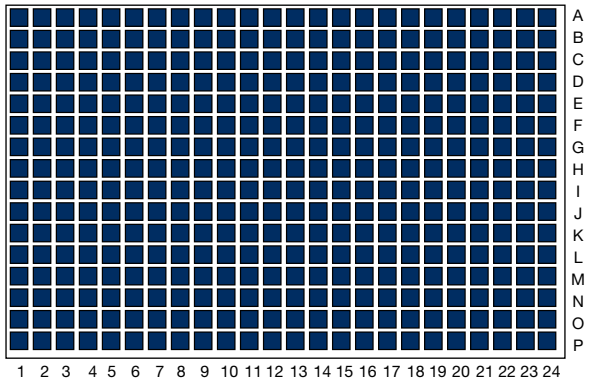


2e Competitor A2 – 96 wells, 2000 µl

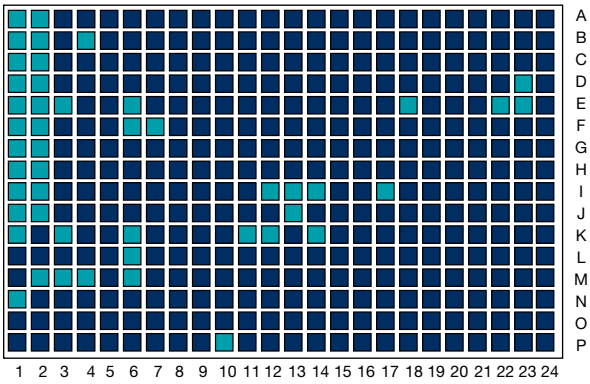


Figures 2 a–e: Depiction of residual volumes per well in all deepwell plates of the 96 well format, 2000 µl, tested. Volume ranges are represented by different colours as indicated in the legend.

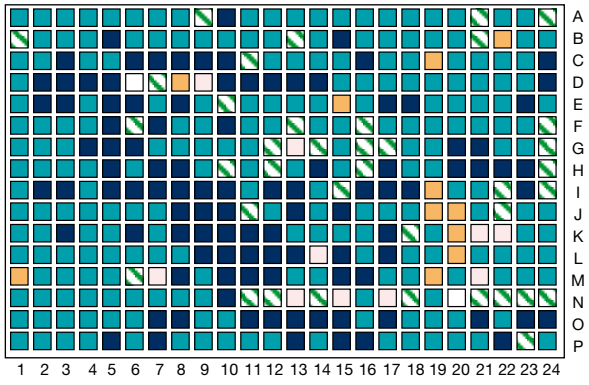
3a Eppendorf Deepwell Plate 384/200 µl



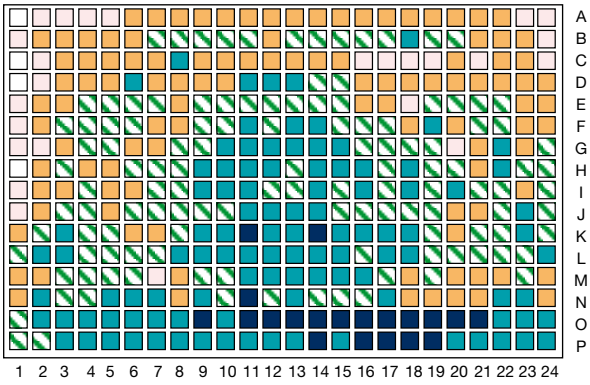
3b Competitor C – 384 wells, 200 µl



3c Competitor A2 – 384 wells, 200 µl



3d Competitor G – 384 wells, 200 µl



Legend

Figures 3 a–d: Depiction of residual volumes per well in all deepwell plates of the 384 well format, 200 µl, tested. Volume ranges are represented by different colours as indicated in the legend.

Conclusion

The data presented in this report verify the functional design and high production quality of the Eppendorf Deepwell Plates. The RecoverMax well geometry ensures sample collection directly at the bottom of the well, where it can be recovered easily. These unique features contribute to the efficient utilization of precious samples as well as expensive reagents. Careful selection of raw materials, combined with an optimized production process, result in plates whose extremely tight tolerances and level well bottom planes render them ideally suited for automated processes.

References

[1] Eppendorf Application Note 146: Eppendorf Plate® Deepwell 96 und 384: g-Safe® – Investigating stability during centrifugation of Eppendorf Plate Deepwell (http://www.eppendorf.com)

[2] Eppendorf Application Note 145: Eppendorf Plate® Deepwell 96 und 384: RecoverMax® – Investigation into the impact of an optimized well design on resuspension properties, sample losses and contamination effects (http://www.eppendorf.com)

[3] Userguide ep*Motion* 5070/5075 No. AU005: Minimization of remaining volumes in plates and tubes (http://www.eppendorf.com).

Ordering information

Eppendorf Deepwell Plate 384/200 µl*/Regular package**				
Quality	**Color*** **	**Packaging**	**Order no. International**	**Order no. North America**
Standard	White	40 plates (5 bags of 8)	0030 521.102	951031003
Sterile	White	40 plates (5 bags of 8)	0030 522.109	951031101
DNA LoBind (also for RNA & other nucleic acids)	White	40 plates (5 bags of 8)	0030 523.105	951031208
Protein LoBind	White	40 plates (5 bags of 8)	0030 524.101	951031305

Eppendorf Deepwell Plate 96/500 µl*/Regular package**				
Quality	**Color*** **	**Packaging**	**Order no. International**	**Order no. North America**
Standard	White	40 plates (5 bags of 8)	0030 501.101	951031801
Sterile	White	40 plates (5 bags of 8)	0030 502.108	951031901
DNA LoBind (also for RNA & other nucleic acids)	White	40 plates (5 bags of 8)	0030 503.104	951032000
Protein LoBind	White	40 plates (5 bags of 8)	0030 504.100	951032107

Eppendorf Deepwell Plate 96/1000 µl*/Regular package**				
Quality	**Color*** **	**Packaging**	**Order no. International**	**Order no. North America**
Standard	White	20 plates (5 bags of 4)	0030 501.209	951032603
Sterile	White	20 plates (5 bags of 4)	0030 502.205	951032701
DNA LoBind (also for RNA & other nucleic acids)	White	20 plates (5 bags of 4)	0030 503.201	951032808
Protein LoBind	White	20 plates (5 bags of 4)	0030 504.208	951032905

Eppendorf Deepwell Plate 96/2000 µl*/Regular package**				
Quality	**Color*** **	**Packaging**	**Order no. International**	**Order no. North America**
Standard	White	20 plates (5 bags of 4)	0030 501.306	951033405
Sterile	White	20 plates (5 bags of 4)	0030 502.302	951033502

*All Deepwell-Plates are available with bar code upon request.
**Also available as bulk pack (384/200 µl and 96/500 µl = 120 plates; 96/1000 µl and 96/2000 µl = 80 plates).
***Available in five color codes (white, yellow, red, green, blue).

Eppendorf ep*Motion*		
Product	**Order no. International**	**Order no. North America**
ep*Motion* 5070, 200–240 V, 50/60 Hz	5070 000.000	960000005
ep*Motion* 5075 LH, 230 V (Liquid handling)	5075 000.008	960020006
ep*Motion* 5075 VAC, 230 (With integrated vacuum station)	5075 000.016	960020014
ep*Motion* 5075 MC, 230 V (Mastercycler ep not included)	5075 000.032	960020303

eppendorf
In touch with life

Your local distributor: www.eppendorf.com/worldwide
Eppendorf AG · 22331 Hamburg · Germany · Tel: +49 40 538 01-0 · Fax: +49 40 538 01-556 · E-Mail: eppendorf@eppendorf.com
Eppendorf North America, Inc. · One Cantiague Road · P. O. Box 1019 · Westbury, N.Y. 11590-0207 · USA
Tel: +1 516 334 7500 · Toll free phone: +1 800 645 3050 · Fax: +1 516 334 7506 · E-Mail: info@eppendorf.com

Application Support Europe, International: Tel: +49 1803 666 789 · E-Mail: support@eppendorf.com
North America: Tel: +1 800 645 3050 ext. 2258 · E-Mail: support_na@eppendorf.com
Asia Pacific: Tel: +60 3 8023 6869 · E-Mail: support_asiapacific@eppendorf.com

Order No. AA18 4WW 020/GB1/WEB/0408/NEUH