Applications

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Eppendorf Plate® Deepwell 96 and 384: RecoverMax®

Investigation into the impact of an optimized well design on resuspension properties, sample losses and contamination effects

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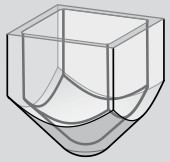
Abstract

The new Eppendorf Plate Deepwell 96 and 384 were developed to make processes of sample preparation, transport and storage more efficient, safe and convenient. The investigation described here demonstrates that the unique RecoverMax design of the wells leads to significantly reduced sample losses and improved yields. There are no irritating "dirty edges" and wicking effects in square wells, and sample preparation and processing are more efficient because processes such as mixing solutions and resuspending pellets are quicker and more complete.

RecoverMax design

1. Improved yields and reduced sample losses
2. No wicking effects and "dirty edges" in square wells
3. Quick and complete mixing and resuspension
4. Efficient sample preparation and processing



Introduction

Mixing of samples and resuspension of sediments and pellets are integral components of most methods in molecular biology and biochemistry. In the age of genomics and proteomics, the processes of bacterial culture, nucleic acid isolation and protein precipitation, for example, are often no longer performed in individual micro tubes, but in 96-well or 384-well plate formats. The high sample throughput required in these research fields or in high-throughput screening in the pharmaceutical industry can only be realized using so-called multi-well plates.
In order to be able to use the sample material obtained effectively and to achieve high yields, it is important for the user that pellets or sediments are dissolved both quickly and completely and that valuable sample material does not accumulate in corners and along edges to be wasted. Wicking effects are also unwanted symptoms of working with multi-well plates: capillary forces frequently draw liquid upwards at the edge of square-shaped wells. This can even lead to the contamination of adjacent wells. The samples of these wells are then usually unusable and valuable material is wasted. This is why Eppendorf developed a new generation of deepwell plates with a special well design, called RecoverMax. Sharp-edged contours were avoided and all the transition points at the walls and bottoms of the wells were smoothed off. The bases are conical in shape and rounded. This causes sample liquids to accumulate at the lowest point of the wells, making pipetting, resuspension and mixing processes quicker and more complete. All the edges, corners and transition points of the square wells of Eppendorf Plate Deepwell 96/2000 µl and 384/200 µl have been rounded off. The settlement of sample material in corners and staged transition points from the well bottom to the well walls ("dirty edges") and the risk of wicking contaminating adjacent wells have now become things of the past.

This application note demonstrates the effect the special RecoverMax design of the Eppendorf Plate Deepwell has on common applications, such as mixing and resuspension as well as the wicking effect and "dirty edges" in square wells.

Materials and methods

1. Mixing sucrose pellets

In order to examine the mixing and resuspension properties of Eppendorf Plate Deepwell, sucrose pellets were coated with an indicator solution (bromothymol blue) and then mixed. As the pellets became increasingly resuspended, the color changed from blue to green to yellow. The color change to yellow indicated complete resuspension of the pellet [1]. Eppendorf Plate Deepwell 96/1000 µl and 384/200 µl formats and plates of the same formats from other suppliers were examined.

Sucrose solution: 2 g/ml sucrose, 0.1 M MES pH = 4.0
Indicator solution: 0.04 mg/ml bromothymol blue, 2 mM Carbonate buffer pH = 10.0

Deepwell plates of the 96/1000 µl format were filled with 50 µl sucrose solution; deepwell plates of the 384/200 µl format, with 5 µl sucrose solution. The plates were then dried for 4 h at 70 °C. The pellets were then coated with 500 µl (96/1000 µl plates) or 50 µl (384/200 µl plates) indicator solution. The pellets were resuspended in the Eppendorf MixMate® at 2000 rpm. The color changed from blue to yellow in a pH range from 6.0 to 7.6.

2. Resuspension of bacterial pellets

150 ml LB medium (Roth, Germany) were inoculated with a strain of *E. coli* K12 bacteria (DH5α) and incubated overnight at 37 °C with shaking. The bacterial suspension was distributed on Eppendorf Plate Deepwell 96/2000 µl and 384/200 µl (see test conditions) and the plates were then pelleted in the Eppendorf Centrifuge 5804 R with Rotor A-2-DWP (deepwell plate rotor). The centrifugation parameters here corresponded to the standard values described in the references. Once the supernatant had been discarded, resuspension buffer (50 mM Tris HCl pH 8.0, 10 mM EDTA, 100 mg/L RNase A) was added to the bacterial pellets (150 µl for 96-well DWP; 30 µl for 384-well DWP). Deepwell plates were then inserted directly into the plate holder of the MixMate and mixed (2000 rpm). A visual check that the pellet was completely resuspended was made after 15 s, 30 s, 45 s, 1 min, 2 min and 5 min.

3. Mixing high-salt buffers

Eppendorf Plate Deepwell 96/1000 µl were filled with 5 µl high-salt buffer and 500 µl dilution buffer per well and mixed in the MixMate at 1,400 rpm. Eppendorf Plate Deepwell 384/200 µl were filled with 1 µl high-salt buffer and 50 µl of dilution buffer per well and mixed in the MixMate at 2000 rpm.

High-salt buffer: 30 % NaCl, 0.1 % (w/v) Patent Blue V
Dilution buffer: 10 mM Tris HCl pH 7.4

4. Wicking

Eppendorf Plate Deepwell 96/2000 µl and comparable deepwell plates from competitors were filled with 1 ml per well of the following buffer solutions and left to stand for 10 min.

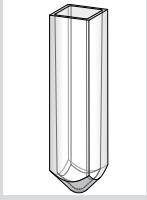
A) Tris HCl 10 mM pH 8.0, Ponceau 4R 0.01 %
B) Tris HCl 10 mM pH 8.0 + 0.01 % Triton® X-100 Ponceau 4R 0.01 %
C) Ponceau 4R 0.1 % in DMSO (100 %)

5. "Dirty edges"

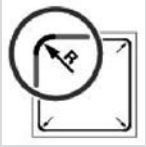
Water colored with Ponceau 4R was pipetted into an Eppendorf Plate Deepwell 96/2000 µl and into corresponding deepwell plates from other suppliers and removed again by pipetting. Residues in the corners and transition points were photographed.

Test conditions bacterial pellets

Eppendorf Plate Deepwell 96/2000 µl
- 1.25 ml overnight culture
- Pellet: 5 min at 1,900 x g
- 150 µl resuspension buffer



Eppendorf Plate Deepwell 384/200 µl
- 200 µl overnight culture
- Pellet: 5 min at 2,200 x g
- 30 µl resuspension buffer



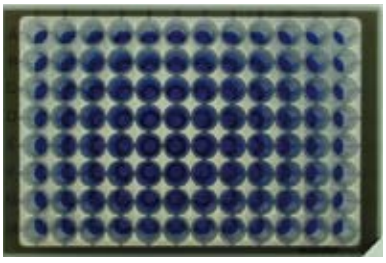
Results and discussions

Resuspension of sucrose pellets

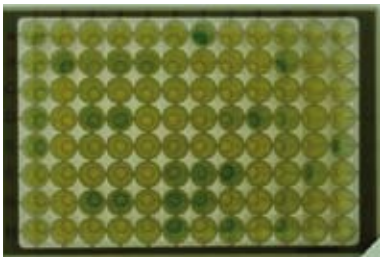
Eppendorf Plate Deepwell 96/1000 µl and 384/200 µl were filled with a sucrose solution and dried. The pellets that formed were coated with an indicator solution (bromothymol blue) and resuspended by mixing in the MixMate. A color change from blue to green to yellow indicated complete resuspension of the pellet. **Figure 1** demonstrates that the color had changed from blue to yellow in the Eppendorf Plate Deepwell 96/1000 µl in almost all the wells after only 5 s. The sucrose pellet was accordingly completely resuspended after just 5 s. Only in a few wells was resuspension still incomplete so that a slight green color could still be observed. In the wells of the competitors' plates A and B, on the other hand, the sucrose pellet was much more difficult to dissolve. After 5 s of mixing, the solutions in the wells were mostly still blue, only a few wells were green in color. Only after 30 s of mixing were the pellets dissolved here too, allowing the complete color change to yellow to be observed.

Eppendorf Plate Deepwell 96/1000 µl

0 s



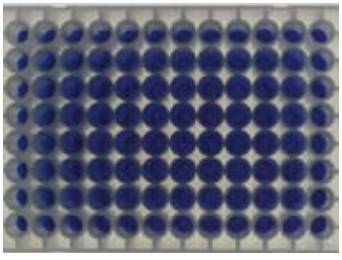
5 s



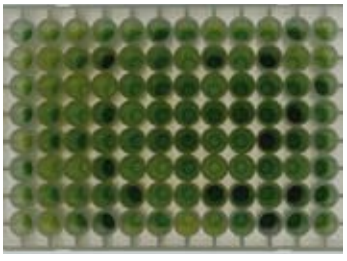
30 s

Competitor A

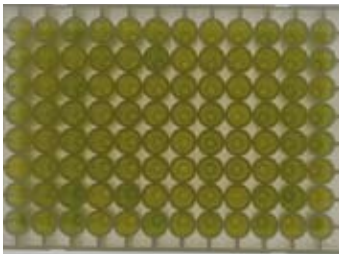
0 s



5 s

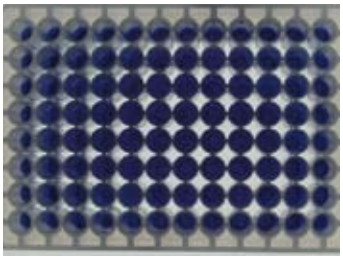


30 s

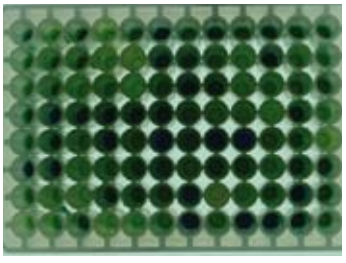


Competitor B

0 s



5 s



30 s

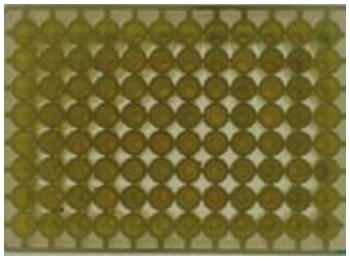
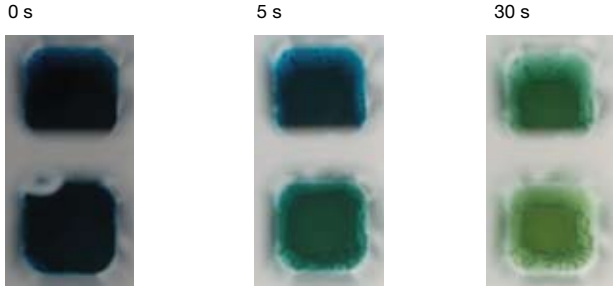


Fig. 1: Resuspension of sucrose pellets with bromothymol indicator solution in an Eppendorf Plate Deepwell 96/1000 µl and in two corresponding deepwell plates from other suppliers. The plates were photographed immediately after the addition of the indicator solution (0 sec), after 5 s of mixing and after 30 s of mixing in the MixMate at 2000 rpm.

Eppendorf Plate Deepwell 384/200 µl

Competitor A



Competitor B

Fig. 2: Resuspension of sucrose pellets with bromothymol indicator solution in an Eppendorf Plate Deepwell 384/200 µl and in two corresponding deepwell plates from other suppliers. The plates were mixed immediately after the addition of the indicator solution (0 s) in the MixMate at 2000 rpm and photographed after 5 s and 30 s

Especially with square wells and at small volumes, it is often difficult to achieve efficient mixing or resuspension of pellets. **Figure 2**, however, shows that the RecoverMax design of Eppendorf Plate Deepwell with square wells leads to efficient resuspension of pellets, even at very small volumes. In the Eppendorf Plate Deepwell 384/200 µl, the color change from blue to yellow was thus complete after only 5 s and the sucrose pellet completely resuspended. In this test, one of the competing plates did comparatively well. However, there was almost no color change observed in competitor plate A even after 30 s and the pellet was accordingly hardly resuspended.

Resuspension of bacterial pellets

The resuspension of solid cell pellets is a common application and integral component of many laboratory methods in biochemistry and molecular biology. Our investigation into the resuspending of bacterial pellets shows that it was possible to resuspend completely the pellet of an overnight culture (1.25 ml per well) in an Eppendorf Plate Deepwell 96/2000 µl within just 30 s (MixMate 2000 rpm) using resuspension buffer (150 µl) (not shown). In plate format 384/200 µl, 200 µl overnight culture per well were dissolved completely in just 30 µl resuspension buffer within only 45–60 s (MixMate, 2000 rpm, not shown). It is clear in this case, too, that the RecoverMax design makes an effective contribution to processing samples quickly and allowing them to be used without waste [2].

Mixing buffers with high salt concentrations

High-salt buffers are used for DNA precipitation, for example. The difficulty here is that a high-density buffer has to be able to be mixed efficiently with a dilution buffer in the wells of deepwell plates. Our investigation into this shows that in the wells of an Eppendorf Plate Deepwell 96/1000 µl, 5 µl of high-salt buffer are completely mixed with 500 µl of the dilution buffer within only 5 s (MixMate 1,400 rpm, not shown). In Eppendorf Plate Deepwell 384/200 µl, it was possible to mix 1 µl high-salt buffer completely in the wells with just 50 µl dilution buffer in 30 s (MixMate 2000 rpm, not shown) [3].

Wicking and cross-contamination

A specific problem when working with square wells is the so-called wicking effect; capillary forces cause sample liquid to rise in the corners of square wells and even reach adjacent wells by this means. Sealing materials may become wet, likewise, increasing the risk of contamination of adjacent wells. Rounding edges and corners and smoothing all transition points in the square wells of Eppendorf Plate Deepwell 96/2000 µl and 384/200 µl should significantly reduce this wicking effect. In order to test the effect of the RecoverMax well geometry on the wicking effect, we examined various buffer mixtures in an Eppendorf Plate Deepwell 96/2000 µl and a corresponding competing plate **(Fig. 3)**.

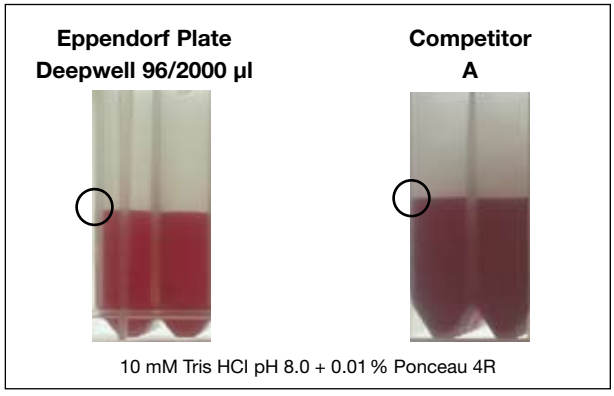


Fig. 3: Wicking effect (circled) in an Eppendorf Plate Deepwell 2000 µl and a corresponding competitor's plate.

In the presence of the Tris HCl buffer, both the Eppendorf Plate Deepwell 96/2000 µl and the competitor's plate had no wicking effect.

The risk of wicking is much higher in buffers containing detergents. **Figures 4 and 5** show that a clear wicking effect was a characteristic of the competitor's plate, while in the Eppendorf Plate Deepwell, only an incipient effect was detectable as a result of the meniscus that forms easily.

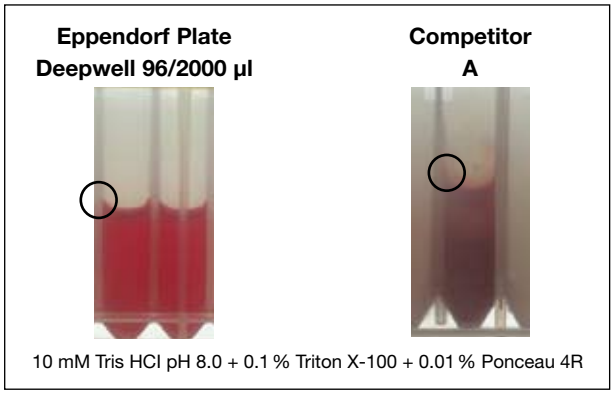


Fig. 4: Wicking effect (circled) in an Eppendorf Plate Deepwell 2000 µl and a corresponding competitor's plate.

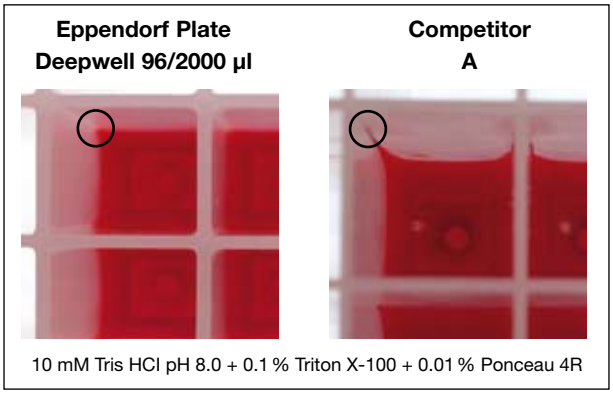


Fig. 5: Wicking effect (circled) in an Eppendorf Plate Deepwell 2000 µl and a corresponding competitor's plate (view from above).

No wicking effect was observed in the wells of an Eppendorf Plate Deepwell filled with DMSO either, whereas the solvent clearly rose in the corners of the competitor's plate **(Fig. 6)**. The results show that the wicking effect and thus the risk of contamination of adjacent wells is stopped by the special well geometry of the Eppendorf Plate Deepwell.

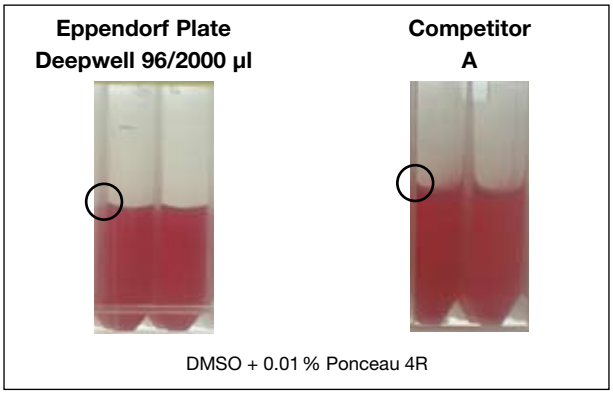


Fig. 6: Wicking effect (circled) in an Eppendorf Plate Deepwell 2000 µl and a corresponding competitor's plate.

Dirty edges

Another phenomenon that occurs in square wells is the settlement of sample material in the corners of the transition points from the base to the wall of the well. Rounding these corners and transition points in the square wells of Eppendorf Plate Deepwell 96/2000 µl and 384/200 µl should avoid such irritating sample settlement, considerably reducing liquid and sample losses. **Figure 7** shows a comparison between an Eppendorf Plate Deepwell 96/2000 µl and two corresponding competitors' plates. Considerable settlement of the buffer could be clearly seen in the well corners in both competitors' plates. The Eppendorf Plate Deepwell, on the other hand, had no buffer settlement.

Eppendorf Plate Deepwell 96/2000 µl **Competitor A** **Competitor B**

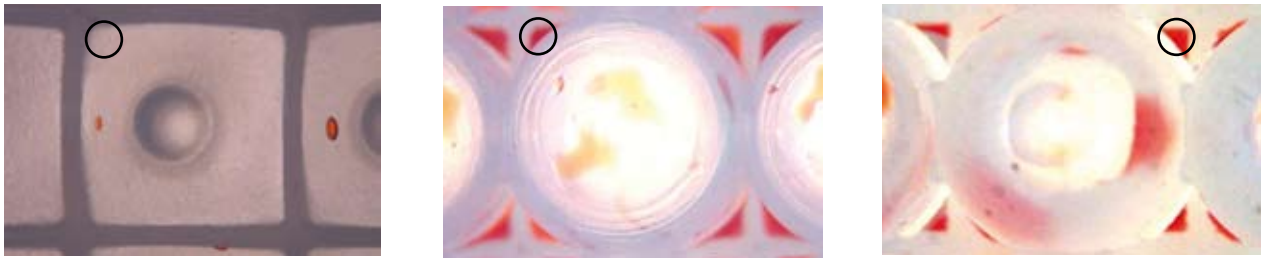


Fig. 7: Eppendorf Plate Deepwell 96/2000 µl and corresponding plates from other suppliers were filled with colored (Ponceau 4R) water and the solution removed again. Critical areas are circled.

Summary

Our investigation into the RecoverMax design of the Eppendorf Plate Deepwell have shown that this optimized well geometry has a strong influence both on resuspension/mixing processes of solid pellets and buffers and on contamination risks from the wicking effect/sample losses due to "dirty edges".

The rounded and smoothed design enables a great deal of time to be saved at a high plate throughput, as resuspension and mixing processes are highly efficient. Sample losses are avoided, as pellets can be dissolved completely and no "dirty edges" form in square wells. Contamination risks such as those caused by the wicking effect which can likewise lead to sample losses are avoided. The use of Eppendorf Plate Deepwell accordingly leads to enormous savings in terms of effort, time and expense, especially at high plate throughput, and gives the user a high level of security against sample wastage.

References

[1] Oldenburg K, Pooler D, Scudder K, Lipinski C and Kelly M. High Throughput Sonication: Evaluation for Compound Solubilization. MatriCal, Inc., Spokane, WA; Pfizer Global Research and Development, Groton, CT.

[2] Osterhoff C, Mueller P, Borrmann L. Eppendorf MixMate – Resuspension of bacteria pellets in deepwell plates (96-and 384-well) and micro test tubes. Eppendorf Application Note 131, 2006.

[3] Osterhoff C, Mueller P, Borrmann L. Comparison of mixing performance in 96- and 384-well plates of Eppendorf MixMate and competitor devices. Eppendorf Application Note 130, 2006.

Ordering information

Eppendorf Plate Deepwell 384/200 µl*

Name	Quality	Color**	Packaging	Order no. international	Order no. North America
Regular package	Standard	red	40 plates (5 bags of 8)	0030 521.129	951031046
	Sterile	red	40 plates (5 bags of 8)	0030 522.125	951031143
	DNA LoBind (also for RNA & other nucleic acids)	red	40 plates (5 bags of 8)	0030 523.121	951031241
	Protein LoBind	red	40 plates (5 bags of 8)	0030 524.128	951031348
Large package	Standard	red	120 plates (10 bags of 12)	0030 525.124	951031445
	Sterile	red	120 plates (10 bags of 12)	0030 526.120	951031542
	DNA LoBind (also for RNA & other nucleic acids)	red	120 plates (10 bags of 12)	0030 527.127	951031640
	Protein LoBind	red	120 plates (10 bags of 12)	0030 528.123	951031747

Eppendorf Plate Deepwell 96/500 µl*

Name	Quality	Color**	Packaging	Order no. international	Order no. North America
Regular package	Standard	red	40 plates (5 bags of 8)	0030 501.128	951031844
	Sterile	red	40 plates (5 bags of 8)	0030 502.124	951031941
	DNA LoBind (also for RNA & other nucleic acids)	red	40 plates (5 bags of 8)	0030 503.120	951032042
	Protein LoBind	red	40 plates (5 bags of 8)	0030 504.127	951032140
Large package	Standard	red	120 plates (10 bags of 12)	0030 505.123	951032247
	Sterile	red	120 plates (10 bags of 12)	0030 506.120	951032344
	DNA LoBind (also for RNA & other nucleic acids)	red	120 plates (10 bags of 12)	0030 507.126	951032441
	Protein LoBind	red	120 plates (10 bags of 12)	0030 508.122	951032549

*All Deepwell plates are available with bar code upon request.
**Available in five frame colors (white, yellow, red, green, blue).

Ordering information

Eppendorf Plate Deepwell 96/1000 µl*

Name	Quality	Color**	Packaging	Order no. international	Order no. North America
Regular package	Standard	red	20 plates (5 bags of 4)	0030 501.225	951032646
	Sterile	red	20 plates (5 bags of 4)	0030 502.221	951032743
	DNA LoBind (also for RNA & other nucleic acids)	red	20 plates (5 bags of 4)	0030 503.228	951032841
	Protein LoBind	red	20 plates (5 bags of 4)	0030 504.224	951032948
Large package	Standard	red	80 plates (10 bags of 8)	0030 505.220	951033049
	Sterile	red	80 plates (10 bags of 8)	0030 506.227	951033146
	DNA LoBind (also for RNA & other nucleic acids)	red	80 plates (10 bags of 8)	0030 507.223	951033243
	Protein LoBind	red	80 plates (10 bags of 8)	0030 508.220	951033341

Eppendorf Plate Deepwell 96/2000 µl*

Name	Quality	Color**	Packaging	Order no. international	Order no. North America
Regular package	Standard	red	20 plates (5 bags of 4)	0030 501.322	951033448
	Sterile	red	20 plates (5 bags of 4)	0030 502.329	951033545
Large package	Standard	red	80 plates (10 bags of 8)	0030 505.328	951033642
	Sterile	red	80 plates (10 bags of 8)	0030 506.324	951033740

*All Deepwell plates are available with bar code upon request.
**Available in five frame colors (white, yellow, red, green, blue).

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