

Applications

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Eppendorf MixMate – Resuspension of bacteria pellets in deepwell plates (96- and 384-well) and micro test tubes

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Abstract

MixMate has been specially developed to mix small volumes in plates (up to 384 wells) and micro test tubes (up to 2.0 ml). Its application range extends from the mixing of liquid sample material to the resuspension of solid cell pellets – an important step in molecular biology research (e.g., in the context of nucleic acid isolation and protein preparation).

The goal of this work was to examine the performance of MixMate in the resuspension of bacteria pellets in various plate and tube formats. The experiments show that Eppendorf MixMate can dissolve bacteria pellets in both 384- and 96-well deepwell plates (DWP), as well as in 1.5 ml and 2.0 ml micro test tubes, in less than 1 min – without any wetting of the tube lids. The results show that, in general, all experimental preparations could be entirely resuspended with a single setting (1 min, 2,000 rpm) with MixMate.

Introduction

As an integral component of methods such as the isolation of plasmid DNA or recombinant proteins, the resuspension of bacteria pellets is one of the most common applications in molecular biology. Complete dissolving of the pellet is, therefore, an important prerequisite for obtaining the optimal yield from the molecules being isolated. However, the resuspension of pellets has often proved to be laborious and time-consuming: for example, in some cases tubes must be individually vortexed, and it is also difficult for some mixers to dissolve the compact bacteria pellets. Some mixers cause wetting of the tube lid because of uncontrolled mixing movements, thus resulting in an increased risk of contamination. Lastly, many plate mixers are not even capable of resuspending bacteria pellets in 96- and 384-well plates.

With MixMate, in contrast, its ^{2D}Mix-Control technology and tube holders can quickly mix both 96- and 384-well

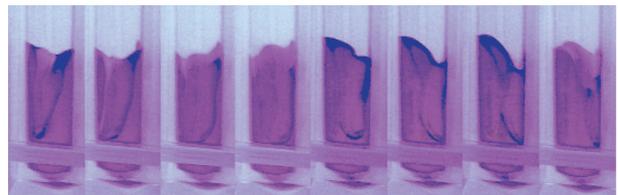


Figure 1: Photographs of the mixing movement in an EppendorfPlate® Deepwell 384/200 using Eppendorf MixMate. The well was filled with 50 µl of water (with Ponceau 4R dye) at a mixing speed of 2,000 rpm. MixMate's ^{2D}Mix-Control technology results in a very effective and controlled circular mixing pattern.

plates (Fig. 1) as well as micro test tubes (1) – without any wetting of the lid or risk of cross-contamination (2). To more precisely examine MixMate's ability to resuspend bacteria pellets bacteria cultures were pelleted in both 96- and 384-well deepwell plates, as well as in both 1.5 ml and 2.0 ml micro test tubes, and then resuspended in buffer.

Materials and methods

150 ml of LB medium (Roth, Karlsruhe, Germany) was inoculated with an *E.coli* K12 bacteria strain (DH5 α) and cultivated overnight under agitation at 37 °C. In accordance with the entries given in table 1, the bacteria suspension was divided among the various plate and tube formats and then pelleted in either Eppendorf® Centrifuge Model 5417 C (for micro test tubes) or Model 5804 R with rotor A-2-DWP (for deepwell plates). The centrifugation parameters (Tab. 1) corresponded to the standard values described in the literature. After disposing of the supernatant, resuspension buffer (50 mM Tris-HCl (pH 8.0), 10 mM EDTA) was added to the bacteria pellets (100 μ l in 1.5 and 2.0 ml micro test tubes; 150 μ l for

96-well DWP; 30 μ l for 384-well DWP). Deepwell plates were subsequently inserted directly into MixMate's universal holder; the micro test tubes were positioned and mixed with the help of the 1.5/2.0 ml tube holder. A visual inspection of the pellet was carried out after 15 s, 30 s, 45 s, 1 min, 2 min and 5 min.

Along with MixMate, Eppendorf Thermomixer comfort (Thermomixer R in the U.S.) with 1.5 ml, 2.0 ml and MTP exchangeable thermoblocks as well as a competitor plate shaker (Competitor A, see also reference 1) were tested. Unless specified otherwise, the experiments were carried out at their maximum mixing speeds.

Results and discussion

Table 1: A comparison of Eppendorf MixMate and two comparable devices for the resuspension of bacteria pellets in micro test tubes and deepwell plates. The times to reach complete resuspension are listed and color-coded (green = pellets resuspended; red = pellets not resuspended after 5 min).

	 1.5 ml micro test tubes	 2.0 ml micro test tubes	 96-well deepwell plates	 384-well deepwell plates (Max. volume: 200 μl)	384-well deepwell plates (Max. volume: 400 μl)
	Test conditions: · 1.5 ml Eppendorf Safe-Lock tube · 1.5 ml overnight culture · Pelleting: 30 s at 16,000 x g · 100 μ l resuspension buffer	Test conditions: · 2.0 ml Eppendorf Safe-Lock tube · 2.0 ml overnight culture · Pelleting: 30 s at 16,000 x g · 100 μ l resuspension buffer	Test conditions: · Eppendorf Plate Deepwell 96/2000 μ l · 1.25 ml overnight culture · Pelleting: 5 min at 1,900 x g · 150 μ l resuspension buffer	Test conditions: · Eppendorf Plate Deepwell 384/200 μ l · 200 μ l overnight culture · Pelleting: 5 min at 2,200 x g · 30 μ l resuspension buffer	Test conditions: · Deepwell Plate 384/400 μ l · 300 μ l overnight culture · Pelleting: 5 min at 2,200 x g · 30 μ l resuspension buffer
MixMate 	45–60 s 2,000 rpm	30 s 2,000 rpm	30 s 2,000 rpm	45–60 s 2,000 rpm	45–60 s 2,000 rpm
Thermomixer comfort 	3 min 1,400 rpm	2 min 1,400 rpm	30 s 1,400 rpm	Not resuspended	Not resuspended
Competitor A*	Mixing of micro test tubes not possible	Mixing of micro test tubes not possible	Not resuspended	Not resuspended	Not resuspended

*More detailed specifications available in Application Note 130.

These experiments show that MixMate resuspends bacteria pellets quickly – and without any wetting of the tube lids. The mixing result is independent of the type of vessel used, and the pellets were dissolved in less than 1 min in 1.5/2.0 ml micro test tubes and 96/384-well deepwell plates (Tab. 1). Results show that all experimental preparations could be entirely resuspended with a single setting (1 min, 2,000 rpm) using Eppendorf MixMate.

When using MixMate's touch vortex function (3,500 rpm mixing frequency), pellets could be entirely resuspended in 1.5/2.0 ml micro test tubes in 10–20 s (results not shown), but there is a risk of lid wetting (and, thus, risk of contamination). It is important to note, however, that

MixMate's tube holders enable the simultaneous mixing of 24 tubes, which easily compensates any time advantage through manual vortexing.

The Eppendorf Thermomixer comfort (Thermomixer R in the U.S.) also demonstrated excellent suitability for the resuspension of bacteria pellets in 1.5/2.0 ml micro test tubes and in 96-well deepwell plates; however, it was primarily developed for the temperature control of samples over long incubation times and therefore takes somewhat longer to completely dissolve pellet. In comparison to MixMate, this device is not particularly suited for resuspension in 384-well plates.

²⁰Mix-Control technology enables MixMate to quickly and efficiently resuspend bacteria pellets – without any wetting of the tube lids – in less than 1 min, both in standard micro test tubes and in 96/384-well plates. Thus it offers the following advantages:

- Time savings – through fast (<1 min) and simultaneous resuspension of up to 24 micro test tubes.
- Reduced risk of contamination – through controlled mixing that prevents wetting of the tube lids.
- Cost savings – eliminates manual pipette tasks and saves on pipette tips
- Increased reproducibility – especially with deepwell plates, as all wells are evenly mixed.
- Flexible application range – accommodates plates and micro test tubes, and features an integrated vortex function.

Literature

- [1] 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.
- [2] Osterhoff C, Mueller P, Borrmann L. Eppendorf MixMate – Experimental evidence of controlled mixing, using a PCR-based chessboard assay. *Eppendorf Application Note 129*, 2006.

Ordering Informations

Description		International order no.	North America order no.
MixMate, incl. 3 tube holders: PCR 96, 0.5 ml, and 1.5/2.0 ml,	230 V, 50–60 Hz 120 V, 50–60 Hz	5353 000.014 5353 000.022	022674200
Eppendorf Plate® Deepwell 96/2000 µl, Standard quality, blue frame, 20 plates (5 sets of 4)		0030 501.349	951033481
Eppendorf Plate® Deepwell 384/200 µl, Standard quality, blue frame, 40 plates (5 sets of 8)		0030 521.145	951031089
Safe-Lock Tubes 1.5 ml, colorless	1,000 pcs. 500 pcs.	0030 120.086 –	– 022363204
Safe-Lock Tubes 2.0 ml, colorless	1,000 pcs. 500 pcs.	0030 120.094 –	– 022363352
Thermomixer comfort (Thermomixer R in the U.S.), without thermoblock,	100–240 V, 50–60 Hz 120 V, 50–60 Hz 220 V, 50–60 Hz	5355 000.011 – –	– 022670107 022670158
Thermoblock for 1.5 ml micro test tubes, incl. IsoRack and IsoPack 0 °C		5360 000.011	022670522
Thermoblock for 2.0 ml micro test tubes, incl. IsoRack and IsoPack 0 °C		5362 000.019	022670549
Thermoblock for MTPs and deepwell plates, with lid		5363 000.012	022670565



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