

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

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## Abstract

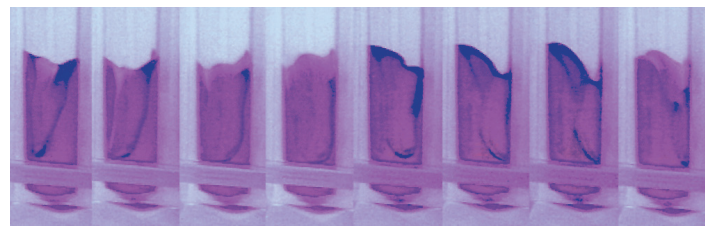
MixMate has been specially developed to mix small volumes in plates (up to 384 wells) and micro test tubes (upto 2.0 ml). Its application range extends from the mixing of liquid sample material to the resuspension of solid cell pellets – an important step in molecular biology research (e.g., in the context 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 timeconsuming: 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 <sup>2D</sup>Mix-Control technology and tube holders can quickly mix both 96- and 384-well plates (Fig. 1) as well as micro test tubes (1) – without any wetting of the lid or risk of cross-contamination.



**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 <sup>2D</sup>Mix-Control technology results in a very effective and controlled circular mixing pattern.

(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 (DH5α) 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 µl in 1.5 and 2.0 ml micro test tubes; 150 µl for 96-well DWP; 30 µl 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, 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

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

**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).

\*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.

**<sup>2D</sup>Mix-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 information

Description		Order no. International	Order no. North America
<b>MixMate®</b> , basic device without tube holders	230 V, 50-60 Hz	5353 000 510	
	110 V, 50-60 Hz	5353 000 529	223100804
<b>Deepwell Plate 96/2000 µL</b> , sterile, blue, 20 plates (5 sets of 4)		0030 501 349	951033588
<b>Deepwell Plate 384/200 µL</b> , PCR clean, white, 10 plates (5 sets of 8)		0030 521 102	951031003
<b>Eppendorf Safe-Lock Tubes 1.5 ml</b> , colorless	1,000 pcs.	0030 120 086	–
	500 pcs.	–	022363204
<b>Eppendorf Safe-Lock Tubes 2.0 ml</b> , colorless	1,000 pcs.	0030 120 094	–
	500 pcs.		022363352
<b>Eppendorf ThermoMixer® C</b> , basic device without thermoblock	220 V – 240 V	5382 000 015	
	100 V – 130 V		5382000023
<b>Eppendorf SmartBlock 1.5 ml</b> , Thermoblock for 24 tubes 1.5 mL		5360 000 038	5360000038
<b>Eppendorf SmartBlock 2.0 ml</b> , Thermoblock for 24 tubes 2.0 mL		5363 000 035	5363000035
<b>Eppendorf SmartBlock plates</b> , Thermoblock for microplates and deepwell plates incl. Lid		5363 000 039	5363000039

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