APPLICATION NOTE No. AA130 I December 2012

Comparison of mixing performance in 96- and 384-well plates of MixMate®, Eppendorf ThermoMixer™ C and competitor devices

Caroline Osterhoff[1], Daniel Dauch[1], Philip Müller[2], Lars Borrmann[1] and Katrin Käppler-Hanno[1]
[1]Eppendorf AG, Hamburg, Germany; [2]Eppendorf Instrumente GmbH, Hamburg, Germany

Abstract

The MixMate® was developed to allow efficient and controlled mixing of small sample volumes from 5 µL to 2 mL in all types of tubes and plates up to 384-well formats. To compare the mixing performance of the MixMate and Eppendorf ThermoMixer™ C with competitor devices, different test samples covering a broad range of possible applications and sample properties (e.g., buffer composition, viscosity, density) were mixed in 96- and 384-well plates. The data presented herein records the mixing duration and visual mixing efficiency of MixMate and Eppendorf ThermoMixer C and 4 competitor devices. The summary chart shows that MixMate and Eppendorf ThermoMixer C outperformed all other instruments in this regards. In combination with the 2-dimensional mixing stroke that eliminates uncontrolled, chaotic movements, MixMate and Eppendorf ThermoMixer C thus enable fast and reliable mixing results and an improved reproducibility of experimental conditions.

Introduction

Nowadays, laboratories are working with much smaller reaction volumes and with higher throughput than they did several years ago. As a consequence, 96- and 384-well plates are becoming more common than ever before. However, these new formats and their special geometries, in combination with reduced reaction volumes, place very specific demands on efficient mixing.
Current findings have shown that efficient mixing of small volumes is not accomplished by simply increasing the mixing speed. Perfect mixing occurs when parameters such as speed, type of mixing movement (e.g., orbital) and the mixing radius are optimized to smoothly run together. Eppendorf has optimized the interplay of all these parameters for the MixMate and the new Eppendorf ThermoMixer C. Termed »^{2D}Mix-Control«, MixMate as well as Eppendorf ThermoMixer C combine 2-dimensional mixing (minimal vertical movements) at high speeds and optimized mixing radius with a reliable plate holder to guarantee rapid and controlled mixing of small volumes (Figure 1, Reference 1).

Fig. 1: Time-lapse photography of controlled mixing with the MixMate: One well of a skirted Eppendorf twin.tec® PCR Plate 96 filled with 75 µL water with dye Ponceau 4R is shown without mixing (left photo) and at 1,650 rpm mixing speed (4 time-lapse photos). Due to the ^{2D}Mix-Control technology, the liquid is forced into an orbital flow without chaotic movements, which enables controlled mixing without wetting the lid.

Introduction

In the experiments described here, we have used different test systems that reflect standard laboratory applications to analyze the mixing performance of MixMate and Eppendorf ThermoMixer C in comparison to competitors devices. Namely the following test systems were used in order to cover a broad range of possible applications and sample properties:

> **Restriction digestion (with and without detergent):** Enzyme storage buffers (e.g., PCR enzymes, restriction enzymes) contain glycerol and partially also detergents such as Triton® X-100 or Tween® 20 to increase enzyme stability during storage at -20 °C. The high density of glycerol and the presence of detergent with its effect on surface tension highly influences mixing behavior.

> **Genomic DNA-assay** to analyze the influence of sample viscosity on mixing performance: In many enzymatic reactions, genomic DNA is used as a template (e.g., PCR amplification, qPCR assays, enzymatic digestion) and thus thorough mixing of the reaction setup is important for reproducible results.

> **Buffers with high salt concentration:** High salt buffers, as for example used for DNA precipitation, were used to analyze the influence of sample density on mixing performance. For this assay, mixing solely depends on the density difference between the high salt buffer and the diluent and is not impaired by any other buffer components, such as protein, glycerol or detergents as can be found within the »restriction digestion« test system.

> **DMSO-containing solutions:** Substances that are not soluble in water (e.g., many proteins, dyes, hormones or antibiotics) are often dissolved in a non-aqueous diluent such as DMSO. As most downstream applications are using aqueous buffer systems, it is necessary to efficiently mix non-aqueous solvents in water-based buffers.

> **Resuspension of bacteria pellets:** The resuspension of compact bacteria pellets highly challenges mixing performance.

As the current trend in science is to use smaller reaction volumes and to perform more reactions in parallel, we have run all experiments with PCR plates, deepwell plates or MTPs with 96- and 384-wells.

Materials and Methods

Composition of buffers used

Diluents:

> Restriction buffer: 1 % glycerol in 10 mM Tris-HCl (pH 8.0)
> Dilution buffer: 10 mM Tris-HCl (pH 7.4)
> Resuspension buffer P1 (Qiagen®, Hilden, Germany): 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 100 mg/L RNase A

Samples:

> Enzyme dummy (without detergent): 5 mg/mL albumin, 20 mM Tris-HCl (pH 7.6), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 50 % glycerol, 0.1 % (w/v) Ponceau 4R
> Enzyme dummy (with detergent): Enzyme dummy (without detergent), 0.01 % Triton® X-100
> gDNA solution: 0.2 µg/µL salmon testis DNA (Sigma-Aldrich®) in TE buffer, 0.1 % (w/v) Patent Blue V
> DMSO solution: 100 % DMSO, 0.1 % (w/v) Patent Blue V
> High salt buffer: 30 % NaCl, 0.1 % (w/v) Patent Blue V
> Bacteria pellet: Bacteria grown from overnight cultures were dispensed into 96- and 384-well deepwell plates. After centrifugation for 5 min at 2,200 x g, the supernatant was removed.

Technical specifications of mixers

Tab. 1: Technical specifications of mixers used. Data were obtained either from the related operating manuals or the homepage of the manufacturer (as of April 2006).

	Maximum rpm	Mixing orbit (diameter)	rpm selection
MixMate	3,000 rpm	3 mm	digital (steps of 50 rpm)
Eppendorf ThermoMixer C	3,000 rpm	3 mm	digital (steps of 50 rpm)
Competitor A	2,800 rpm	1 mm	analog
Competitor C	1,000 rpm	2 mm	analog
Competitor D	2,200 rpm	n.d.	analog
Competitor F	2,500 rpm (max. 50 % with microplate)	4 mm	analog

Results

To compare the mixing performance between the MixMate, Eppendorf ThermoMixer C and 4 competitor devices different sample materials were mixed in 96- and 384-well plates. In order to track the degree of mixing, a visual check was performed after 5 s, 15 s, 30 s, 1 min, 3 min and 5 min of mixing, respectively. The results for these experiments are listed in Table 2.
The results show that MixMate and Eppendorf ThermoMixer C mix all samples efficiently in max. 1 min, independent of the buffer system or the type of plate being used. For most samples, a mixing time of only a few seconds was sufficient to achieve complete mixing. Bacteria cell pellets in 384-well plates were resuspended in max. 2 min (Eppendorf ThermoMixer C) or max. 3 min (MixMate). Interestingly, neither mixers with larger or a smaller mixing orbits (Table 1) perform as well in mixing compared to MixMate and Eppendorf ThermoMixer C at comparable mixing speeds. Thus, mixing performance does not solely depend on the simple combination of mixing speed and mixing orbit. Other parameters such as smooth operation without unnecessary vibrating, sturdiness, stability of the plate holder seem to be essential to guarantee optimal mixing performance for all types of samples and vessel shapes.

Tab. 2: Comparison of mixing performance of MixMate, Eppendorf ThermoMixer C and competitor devices: Different experimental setups reflecting standard laboratory applications were tested in 96- and 384-well plates.

96-well plates	**MixMate®**	**Eppendorf ThermoMixerC™**	**Competitor A**	**Competitor C**	**Competitor D**	**Competitor F**
Restriction digestion (without detergent)	30 s (2,100 rpm)	1 min (2,000 rpm)	not mixed	not mixed	3 min	not mixed
Restriction digestion (with detergent)	15 s (1,800 rpm)	30 s (1,800 rpm)	1 min	not mixed	45 s	not mixed
Mixing of genomic-DNA	30 s (2,000 rpm)	30 s (2,000 rpm)	5 min	not mixed	3 min	not mixed
Mixing of buffers with high salt concentration	5 s (1,400 rpm)	5 s (1,400 rpm)	not mixed	not mixed	1 min	not mixed
Mixing of DMSO-containing solutions	5 s (1,100 rpm)	5 s (1,100 rpm)	3 min	not mixed	15 s	30 s
Resuspension of bacteria pellets	30 s (2,000 rpm)	30 s (2,000 rpm)	not mixed	not mixed	3 min	not mixed
384-well plates	**MixMate**	**Eppendorf ThermoMixer C**	**Competitor A**	**Competitor C**	**Competitor D**	**Competitor F**
Restriction digestion (without detergent)	30 s (3,000 rpm)	30 s (3,000 rpm)	not mixed	not mixed	not mixed	not mixed
Restriction digestion (with detergent)	45 s (2,800 rpm)	30 s (2,800 rpm)	not mixed	not mixed	5 min	not mixed
Mixing of genomic-DNA	45 s (3,000 rpm)	1 min (3,000 rpm)	not mixed	not mixed	5 min	not mixed
Mixing of buffers with high salt concentration	1 min (2,000 rpm)	30 s (2,000 rpm)	not mixed	not mixed	not mixed	not mixed
Mixing of DMSO-containing solutions	5 s (2,200 rpm)	5 s (2,000 rpm)	not mixed	not mixed	3 min	not mixed
Resuspension of bacteria pellets	3 min (2,000 rpm)	2 min (2,000 rpm)	not mixed	not mixed	5 min	not mixed

- efficient mixing in less than 1 min
- mixed within 1 min–5 min
- not completely mixed within 5 min

Discussion

The trend of using 96- or 384-well plates instead of micro test tubes place very specific demands on efficient mixing because of the special vessel geometries and the small reaction volumes used within. The data presented in this Application Note show that the MixMate as well as Eppendorf ThermoMixer C meet all of these demands as it is capable of rapid mixing of samples in 96- and 384-well plates. All tested solutions were mixed efficiently within a few seconds independently from sample properties such as buffer composition, viscosity or density. Even compact bacteria pellets were resuspended efficiently. The innovative ^{2D}Mix-Control technology allows controlled mixing without lid wetting in standard laboratory applications. This saves time by reducing unnecessary centrifugation steps and reduces the risk for cross-contamination [1]. With the unique combination of mixing efficiency and controlled mixing stroke, the Eppendorf devices MixMate, Eppendorf ThermoMixer C, Eppendorf ThermoMixer F1.5 and Eppendorf ThermoMixer FP enable reliable mixing results and guarantee maximum reproducibility of experimental conditions for different types of samples, reaction volumes and plates.

References

[1] 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
Eppendorf ThermoMixer™ C, basic device without thermoblock220 V – 240 V 100 V – 130 V	5382 000.015 –	– 5382000023
Eppendorf ThermoTop®, with **condens.**protect® technology	5308 000.003	5308000003
Lid, for Eppendorf ThermoMixer™ F1.5, Eppendorf ThermoMixer® FP for Eppendorf SmartBlocks™ 0.5 mL, 1.5 mL, 2.0 mL, plates, PCR 96, PCR 384	5363 000.233	5363000233
Eppendorf SmartBlock™ 0.5 mL, Thermoblock for 24 tubes 0.5 mL	5361 000.031	5361000031
Eppendorf SmartBlock™ 1.5 mL, Thermoblock for 24 tubes 1.5 mL	5360 000.038	5360000038
Eppendorf SmartBlock™ 2.0 mL, Thermoblock for 24 tubes 2.0 mL	5362 000.035	5362000035
Eppendorf SmartBlock™ 5.0 mL, Thermoblock for 8 tubes 5.0 mL	5309 000.007	5309000007
Eppendorf SmartBlock™ 15 mL, Thermoblock for 8 conical tubes 15 mL	5366 000.021	5366000021
Eppendorf SmartBlock™ 50 mL, Thermoblock for 4 conical tubes 50 mL	5365 000.028	5365000028
Eppendorf SmartBlock™ 12 mm, Thermoblock for 24 tubes diameter 11 mm – 11.9 mm, height 34 mm – 76 mm	5364 000.024	5364000024
Eppendorf SmartBlock™ cryo, Thermoblock for 24 Cryo containers 1.5 mL – 2 mL, diameter max. 12.5 mm, all base shapes	5367 000.025	5367000025
Eppendorf SmartBlock™ plates, Thermoblock for microplates and deepwell plates incl. Lid	5363 000.039	5363000039
Eppendorf SmartBlock™ PCR 96, Thermoblock for PCR plates 96 incl. Lid	5306 000.006	5306000006
Eppendorf SmartBlock™ PCR 384, Thermoblock for PCR plates 384 incl. Lid	5307 000.000	5307000000
MixMate®, includes 3 tube holder; PCR 96, 0.5 mL, 1.5/2.0 mL, 230 V, 50–60 Hz 120 V, 50–60 Hz	5353 000.014 5353 000.022	 022674200
Eppendorf twin.tec® PCR Plate 96, skirted (clear wells), Clear frame, 25 pcs.	0030 128.648	951020401
Eppendorf twin.tec® PCR Plate 96, semi-skirted (clear wells), Clear frame, 25 pcs.	0030 128.575	951020303
Eppendorf twin.tec® PCR Plate 384 (clear wells), Clear frame, 25 pcs.	0030 128.508	951020702
Eppendorf Deepwell plate™ 96/1000 µL, Standard quality, Blue frame, 20 plates (5 bags of 4)	0030 501.241	951032689
Eppendorf Deepwell plate™ 96/2000 µL, Standard quality, Blue frame, 20 plates (5 bags of 4)	0030 501.349	951033481
Eppendorf Deepwell plate™ 384/200 µL, Standard quality, Blue frame, 40 plates (5 bags of 8)	0030 521.145	951031089
PCR Film (self-adhesive), 100 pcs.	0030 127.811	951023019

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