

Determining the Optimized Mixing Parameters of Your Samples Using Eppendorf ThermoMixer® C

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Abstract

Homogenization ensures that all components of the sample are evenly distributed. This uniformity is essential for accurate and reproducible results, as it guarantees that every part of the sample has the same concentration of reactants. The Eppendorf ThermoMixer® C is designed to provide optimized mixing performance for a variety of laboratory applications. This application note evaluates the mixing efficiency of the Eppendorf ThermoMixer C using different challenging test systems, including enzyme reactions, genomic DNA, buffers with high salt concentration and the resuspension of bacteria pellets. To analyze the mixing efficiency two methods introducing reference samples and measuring absorbance readings were developed. The obtained results illustrate how time, filling level and format of the reaction vessel can influence mixing efficiency. The results demonstrate the capability of the ThermoMixer C to achieve fast and effective homogenization across a wide range of sample types and volumes, ensuring reproducible and reliable results in laboratory workflows.



Introduction

Optimal mixing occurs when parameters such as speed, type of mixing movement (e.g., orbital) and the mixing radius are optimized to smoothly run together. For many samples it is hard to determine by a visual observation if a sample is completely mixed. By introducing a reference sample, the mixing could be evaluated based on absorbance readings.

In the experiments described here, we have used different test systems that reflect standard laboratory applications to optimize the mixing performance. The developed method as well as the determined parameters provide a guidance to achieve effective homogenization of challenging samples.

Enzyme reaction:

Enzyme storage buffers (e.g., PCR enzymes, restriction enzymes) contain glycerol and partially also detergents such as dithiothreitol (DTT) 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 as it has a higher viscosity and density than water.

Genomic DNA:

In many enzymatic reactions, genomic DNA is used as a template (e.g., (q)PCR assays, hybridization assays, enzymatic digestion, mRNA synthesis) and thus mixing of the genomic DNA 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 Enzyme reaction test system.

Resuspension of bacteria pellets:

The resuspension of compact bacteria pellets highly challenges mixing performance mainly due to the high density, compactness and adhesion of the cells.

Materials and Methods

Composition of samples and buffers used

Enzyme reaction:

Enzyme dummy: 5 mg/mL Albumin, 20 mM TRIS-HCl (pH 8), 100 mM KCl, 1 mM EDTA
1 mM DTT, 50% Glycerin, 0.1% (w/v) Cochenille red (Ponceau 4R)
Reaction buffer: 20 mM TRIS-HCl (pH 8)

Genomic DNA:

Genomic DNA solution: 0.2 mg/ml herring sperm-DNA in 1 x TE buffer, 0.1% (w/v) Patent Blue VF
Dilution buffer: 10 mM TRIS-HCl (pH 7.4)

Buffer with high salt concentration:

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

Resuspension of bacteria pellets:

E. coli bacteria pellet:
Bacteria growth in LB media from overnight cultures, centrifugation 5 min at 3,700 x g
Resuspend solution: LB medium

Determine the mixing performance in the homogenization of solutions

Reaction vessels were filled with the buffer and the test solution containing the dye was pipetted carefully onto the bottom of the vessels. The reference sample was perfectly homogenized by pipetting 50% of the volume up and down ten times. Afterwards, the absorbance of the reference sample was determined (Cochénille red at 505 nm and Patent Blue

VF absorbance at 640 nm) with a NanoDrop™ 2000 from Thermo Scientific. The optioned value served as the reference value (Step 1 in Figure 1 as an example). The sample was mixed on the Eppendorf ThermoMixer® C until the absorbance reaches the reference value (Step 2 in Figure 1 as an example). Several samples were tested per condition simultaneously in different positions of the respective SmartBlock.

Step 1: Determine the absorbance reference value

Reference sample



NanoDrop

OD505 = x (= reference value)

Step 2: Determine the mixing parameters

Sample



NanoDrop

Mixing up to OD505 reaches the x reference value

Figure 1: Workflow 1 describing the determination of mixing performance for all tested solutions using the workflow for the »Enzyme reaction« in Eppendorf Safe-Lock® Tubes 1.5 ml as an example. Step 1: The enzyme dummy containing Cochénille red and the reaction buffer were mixed by pipetting. Afterwards absorbance readings of the completely mixed solution were performed to determine the reference value. Step 2: The same reactants were mixed on the Eppendorf ThermoMixer C until the absorbance readings reached the reference value.

Determine the mixing performance in bacteria pellet resuspension

An *E. coli* culture was diluted to a OD600 of 0.2. The required volume was dispensed into the appropriate reaction vessel (Figure 2). The exact OD600 of the suspension in the consumable was measured and noted as the reference value. Afterwards, the bacteria cells were pelleted with high speed Centrifuge CR22N for 5 minutes at 5,000 xg (3,700 xg for Deepwell plate). The sample was mixed

with the Eppendorf ThermoMixer C until the OD600 reached the reference value. Several samples were tested per condition simultaneously in different positions of the respective SmartBlock. For the OD600 determination in a Deepwell plate (DWP) 200 µl of the *E. coli* suspension were transferred with epMotion 96 in a reading 96 well plate for measurements in the plate reader xMark™ Microplate Spectrophotometer and then pipette back in the DWP for centrifugation.

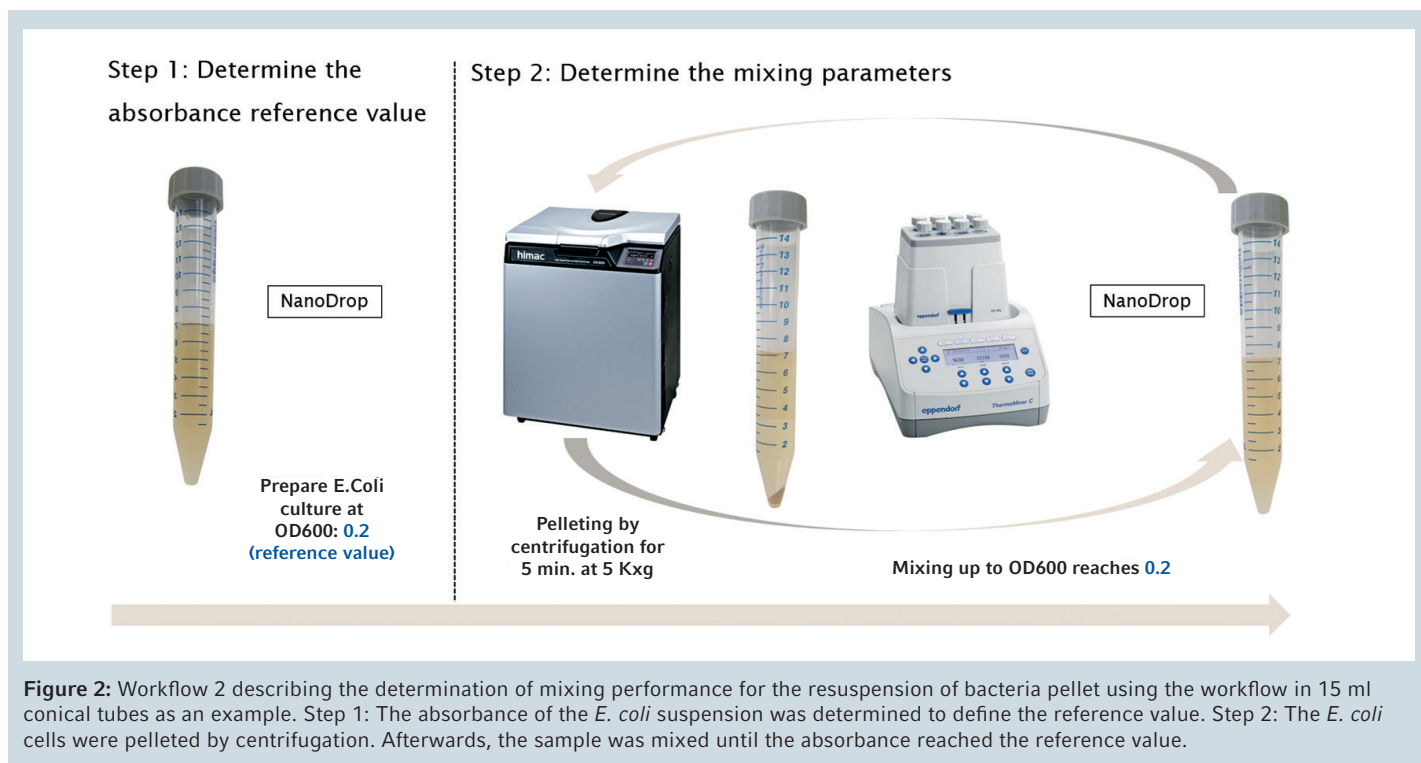


Figure 2: Workflow 2 describing the determination of mixing performance for the resuspension of bacteria pellet using the workflow in 15 ml conical tubes as an example. Step 1: The absorbance of the *E. coli* suspension was determined to define the reference value. Step 2: The *E. coli* cells were pelleted by centrifugation. Afterwards, the sample was mixed until the absorbance reached the reference value.

Results and Discussion

Fast and effective homogenization of samples using the example of enzyme reaction and high salt solution

The process of homogenizing samples quickly and effectively has been demonstrated through two exemplary results for the enzyme reactions and high salt solutions.

In the first example, 200 μ l of an enzyme reaction were homogenized using 1.5 ml tubes and the SmartBlock 1.5 ml

at a speed of 1,800 rpm for 10 seconds (Figure 3A).

Similarly, the homogenization of 800 μ l of a high salt solution was carried out in Eppendorf Deepwell Plate 96/1000 μ l on SmartBlock DWP 1000 at 1,500 rpm in 20 seconds (Figure 3B). No spillage has been observed. In addition, both results illustrated that samples have been homogenized independent of their position on the SmartBlock.

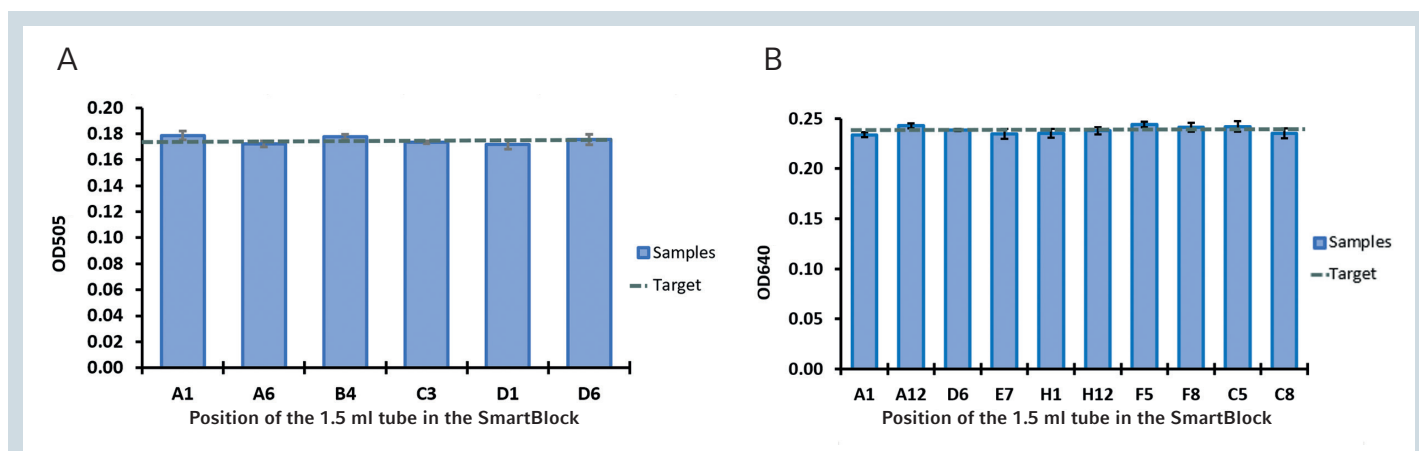


Figure 3: Fast and effective homogenization of different sample types and volumes with Eppendorf ThermoMixer® C. Dashed green lines indicate the reference OD505 (reference value of a control which was completely mixed by manual pipetting). (A) Enzyme reaction: OD505 of 190 μ l enzyme reaction + 10 μ l Cochenille red after mixing in Eppendorf Safe-Lock Tubes 1.5 ml using SmartBlock 1.5 ml at 1,800 rpm for 10 seconds. (B) High salt solution: OD640 of 784 μ l high salt solution + 16 μ l Patent Blue VF after 20 seconds of mixing in Eppendorf Deepwell Plate 96/1,000 μ l on SmartBlock DWP 1000 at 1,500 rpm.

Influence of filling level, time and vessel format on mixing performance

In the following, we would like to discuss three factors besides mixing speed, type of mixing movement and the mixing orbit which can have an impact on the mixing performance. (1) The filling level in a reaction vessel significantly impacts the time required for complete homogenization. For instance, the homogenization of genomic DNA in 1.5 ml tubes at 1,800 rpm (Figure 4) required only 10 seconds for 100 μ l, but 20 and 30 seconds for 500 μ l and 1,500 μ l, respectively. This demonstrates that higher filling levels can require longer mixing times to achieve complete homogenization.

(2) The second factor is time. Time-dependency is illustrated when *E. coli* cell pellets were resuspended in 25 ml conical tubes at 1,000 rpm (Figure 5A). A complete resuspension of the cells was achieved after 60 seconds where the sample reached the tube lid.

(3) The vessel type can also have an important influence. When 50 ml conical tubes were used under the same conditions a complete resuspension could already be achieved after 20 seconds (Figure 5B). The time difference could be probably explained by the fact that the sample did not reach the tube cap when using the 50 ml conical tube which

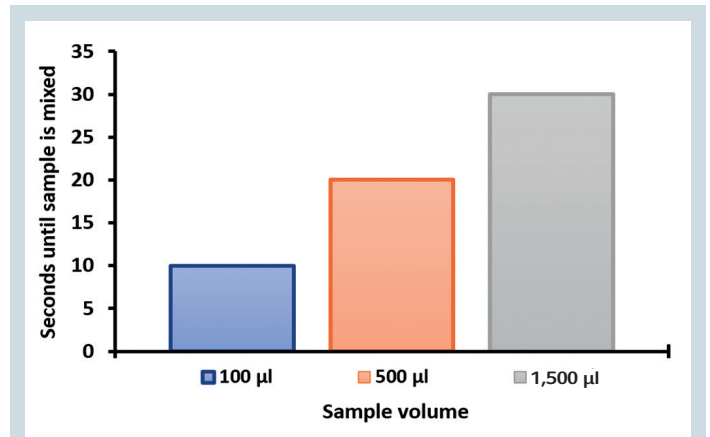


Figure 4: Influence of filling level in the vessel on mixing performance. Patent Blue VF was added to different volumes of genomic DNA solution. The samples were mixed in 1.5 ml tubes at 1,800 rpm. OD640 was measured every ten seconds. Time in seconds until sample was completely homogenized (OD640 of the sample matched reference value).

allows the liquid to be forced into a more circular flow. The usage of the 15 ml conical tube was most time consuming. Here 225 seconds were needed for a complete resuspension of the cells in only 7 ml medium. It can be assumed, that the smaller diameter of the 15 ml conical tube caused negative effects on mixing efficiency.

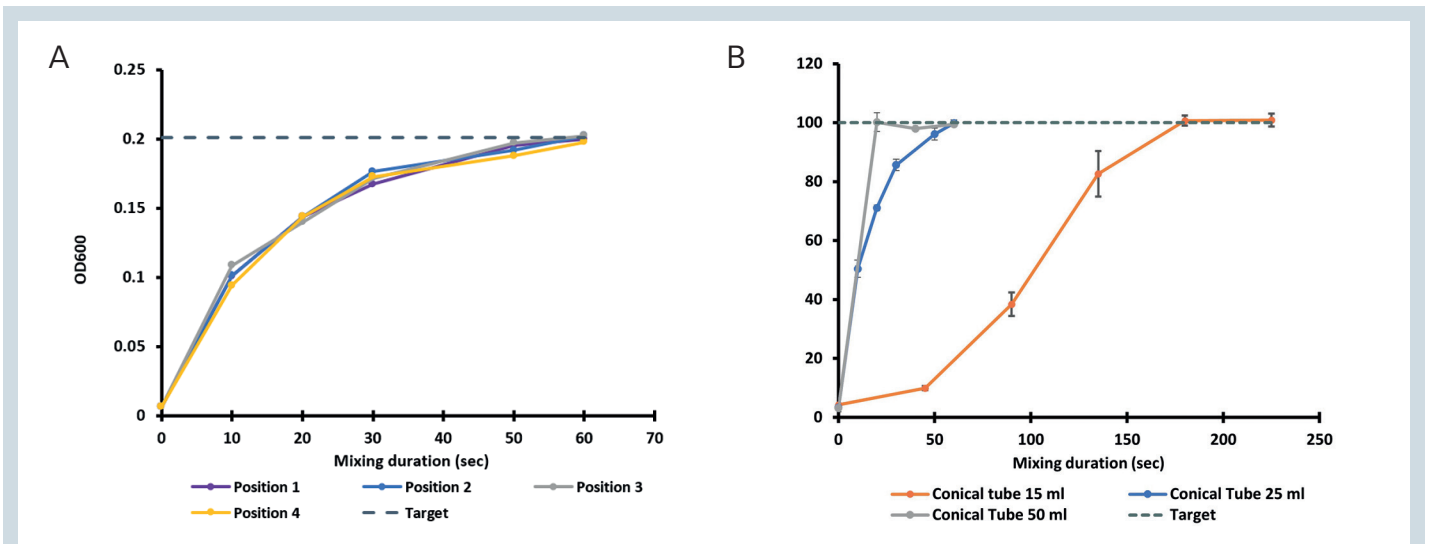


Figure 5: Resuspension of *E. coli* cells in conical tubes. An *E. coli* overnight culture was filled in conical tubes and centrifugated 5 min at 3,700 x g. The bacterial cells were resuspended and OD600 was measured (at least) until the reference value (OD600 of the overnight culture before centrifugation) was reached. (A) Influence of mixing time on sample homogenization. Resuspension of 10 ml bacterial cells in 25 ml conical tubes at 1,000 rpm using SmartBlock 50 ml with adapter 25 mL. The position of the conical in the SmartBlock has no effect on mixing performance. (B) Influence of vessel format on mixing performance. Mean % of reference OD600 when resuspending bacterial cells at 1,000 rpm in conical tubes for 15, 25 and 50 ml.

Conclusion

Complete homogenization of a sample is essential for achieving reliable, accurate, and reproducible results. It ensures uniformity, consistent reaction conditions, and accurate measurement. Here, an approach based on absorbance reading has shown that the Eppendorf ThermoMixer C is an effective tool to optimize mixing performance across various laboratory applications. The device's ability to handle different sample types, from enzyme reactions to high salt buffers and bacterial pellet resuspensions, highlights its versatility and

efficiency. The ^{2D}Mix-Control feature of the ThermoMixer C ensures controlled and consistent mixing without spillage or chaotic movements of the sample. The results and the protocols to optimize mixing parameters provide valuable guidance for researchers to achieve reliable and reproducible results. In addition, the obtained results illustrate that time, filling level and format of the vessel can influence mixing efficiency. Overall, the ThermoMixer C is a robust and reliable solution for various laboratory mixing needs.

Ordering information

Description	Order no.
Eppendorf ThermoMixer® C	5382000015
SmartBlock 1.5 mL	5360000038
SmartBlock 15 mL	5366000021
SmartBlock 50 mL	5365000028
SmartBlock PCR 96	5306000006
SmartBlock Plates	5363000039
SmartBlock DWP 1000	5310000002
High-Speed Centrifuge CR22N	5721220012
epMotion® 96xl	5069000314
Deepwell Plate 96/1000 µl	0030501233
Deepwell Plate 96/2000 µl	0030502310

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