Sample preparation

Improved efficiency and reproducibility using the automated Multidrop Combi Reagent Dispenser

Introduction

Nucleic acid sample preparation is a critical process in molecular biology. It is imperative to be able to efficiently extract high-quality nucleic acid suitable for downstream applications. The use of magnetic bead–based kits for nucleic acid extraction has become commonplace due to their ease of use and reliability of results. The magnetic bead–based kits can be paired with an automated extraction instrument, such as the Thermo Scientific™ KingFisher™ Flex Purification System, suited to the laboratory's desired throughput. The protocols for magnetic bead–based sample preparation kits include steps in which the reagents must be dispensed into the respective 24- or 96-well plates that are placed on the sample preparation platform. The routinely used method of dispensing the magnetic beads and reagents is to manually pipet them into the plates. However, manual liquid handling is tedious, time consuming, prone to human error, and reduces workflow efficiency. One way to circumvent these issues is to use an automated liquid handling platform, such as the Thermo Scientific™ Multidrop™ Combi Reagent Dispenser

The objective of this study was to examine the potential benefits of using the Multidrop Combi dispenser for the preparation of plates for sample extraction on the KingFisher Flex instrument. We evaluated the efficiency and reproducibility of manually dispensing magnetic beads and extraction reagents compared to dispensing them with the Multidrop Combi Reagent Dispenser. Reagents and beads used for this study were from the Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kit and the MagMAX™ Cell-Free DNA Isolation Kit.

Materials and methods

DNA extraction kits evaluated

Sample preparation kits for this study were chosen to cover multiple applications and for the diversity of components within the kits (Table 1).

Table 1. Kit specifications, sample parameters, and applications

	MagMAX DNA Ultra 2.0 kit	MagMAX Cell-Free DNA kit
Cat. No.	A45721	A29319
Sample type	Saliva	Plasma
Sample stabilizer	SpeciMAX nucleic acid stabilization solution	K2 EDTA
Sample volume for this study (per well)	200 µL	2 mL and 4 mL
Plate type	96 deep-well	24 deep-well
No. of samples run for this study	288	72
Applications	SARS-CoV-2, microarray, sequencing, human identification, genotyping	Oncology, NGS

The MagMAX DNA Multi-Sample Ultra 2.0 and MagMAX Cell-Free DNA Isolation Kits are used in a wide range of research areas such as genomics, genetic research, and oncology. The isolated nucleic acids are used for downstream applications including quantitative PCR (qPCR), microarray, and next-generation sequencing (NGS). Both MagMAX DNA isolation kits use unique buffer chemistries and magnetic beads. The MagMAX DNA Multi-Sample Ultra 2.0 Kit offers both 96- and 24-well extraction options suited to the sample type and volume. The MagMAX Cell-Free DNA Isolation Kits are for use with the 24-well plate format due to the large starting sample volumes required for liquid biopsy work.

The input for the MagMAX DNA Multi-Sample Ultra 2.0 Kit was human saliva collected using the Thermo Scientific™ SpeciMAX™ Stabilized Saliva Collection Kit. The input sample for the MagMAX Cell-Free DNA Isolation Kit was cell-free human DNA in K2 EDTA plasma. Saliva (2 mL) was collected from 30 healthy adult donors, and the human plasma was purchased from ProMed DX (North America) and BioIVT (North America). A 96-well format was used to extract DNA from 200 µL of pooled stabilized saliva with the MagMAX DNA Multi-Sample Ultra 2.0 Kit, while a 24-well format was used to extract cell-free DNA (cfDNA) from 2 mL and 4 mL of pooled plasma in K2 EDTA.

Efficiency of extractions

To determine the efficiency of running manually dispensed reagents compared to those using the automated Multidrop Combi dispenser, three replicate plates were prepared for each kit. One-half of the plate was used for manually dispensing, and the other half of the plate was for dispensing using the Multidrop Combi dispenser (Figure 1).

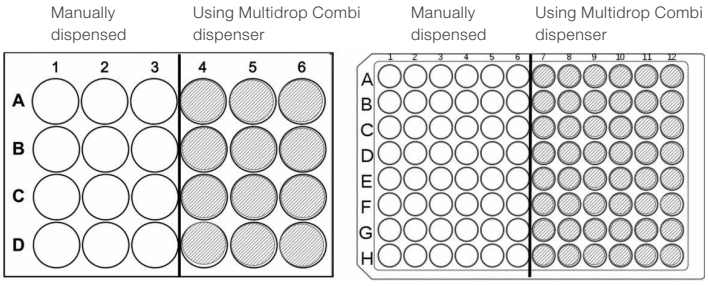


Figure 1. Diagrams of 24 (left) and 96 (right) deep-well plate layouts for dispensing beads and reagents manually (white wells) or with the Multidrop Combi dispenser (gray wells).

A timer was used to measure the total time for each method. For both kits, a mixture of the binding solution and magnetic beads was prepared (including 10% overage) in a bottle. The aspirating tips of the cassette tubes were placed in the bottle for dispensing to ensure the volume exceeded the requirement for the cassette (volume for standard cassette: 12 mL). The beads and binding solution were mixed and dispensed together using the Multidrop Combi dispenser rather than dispensing either reagent alone. All volumes dispensed for wash, ethanol, and elution solution were according to the kit protocol.

Reproducibility of extractions

To determine reproducibility, all saliva and plasma samples were pooled separately prior to extraction to make a homogeneous sample for each specimen type. Duplicate 96-well plates for the MagMAX DNA Ultra 2.0 Kit and triplicate 24-well plates for the MagMAX Cell-Free DNA Isolation Kit were prepared, run on the KingFisher Flex instrument, and eluted in 50 µL volumes. The eluates were checked for purity using a Thermo Scientific™ NanoDrop™ OneC Microvolume UV-Vis Spectrophotometer by calculating the absorbance ratios (A_{260}/A_{280}). Concentrations of the extracted DNA were determined using an Invitrogen™ Qubit™ 1X dsDNA High Sensitivity Assay. To determine the reproducibility of the concentration of DNA extracted using manually dispensed or automatically dispensed reagents, statistical significance testing was performed using the Student's *t*-test. To evaluate the size and intactness of the genomic and cell-free DNA, extracted eluates for each sample type were run on an Agilent™ 2100 Bioanalyzer™ system and the Agilent™ 4200 TapeStation™ system. The 4200 TapeStation software generates a DNA integrity number (DIN) for genomic DNA (gDNA) that reflects the quality of the DNA; the software also generates a percent cell-free DNA value for gDNA samples that reflects the amount of cfDNA to gDNA isolated. To determine the yield of human-specific DNA, qPCR for the human RNase P gene using the Applied Biosystems™ TaqMan™ RNase P Detection Reagents Kit was performed.

Robustness of extractions

To test for the robustness of Multidrop Combi dispensing, extractions from plasma using the MagMAX Cell-Free DNA Isolation Kit were performed in two separate labs by two different technicians and were analyzed on the 2100 Bioanalyzer system or the 4200 TapeStation software.

Results

Efficiency of extractions

The results for three deep-well plates show time savings for both kits when using automated dispensing (Table 2). The average time for dispensing MagMAX Cell-Free DNA beads and reagents in half of the 24-well plate was 2 min 25 sec using the Multidrop Combi dispenser and 6 min 20 sec for manual dispensing. The Multidrop Combi dispensing saved an average of 3 min 55 sec. The average time for dispensing MagMAX DNA Ultra 2.0 beads and reagents in half of the 96-well plate was 1 min 25 sec using the Multidrop Combi dispenser and 5 min 10 sec for manual dispensing. The Multidrop Combi dispenser saved an average of 3 min 45 sec.

Table 2. Average time for dispensing magnetic beads and reagents from the MagMAX kits.

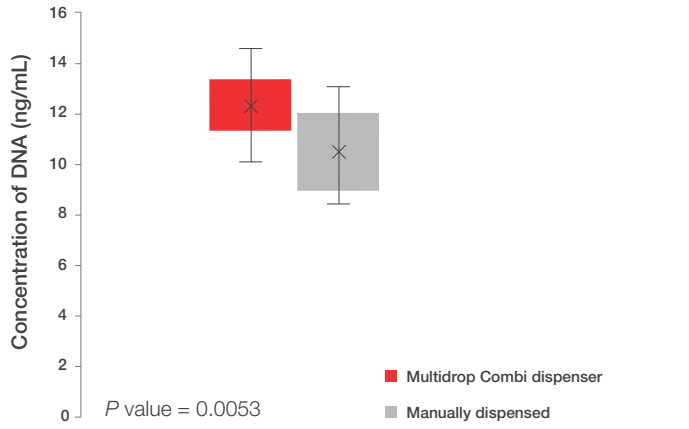
MagMAX Cell Free DNA beads and reagents	Multidrop Combi dispenser	Manual	Time difference
Average for 24 well plates	2 min 25 sec	6 min 20 sec	3 min 55 sec
MagMAX DNA Ultra 2.0 beads and reagents	**Multidrop Combi dispenser**	**Manual**	**Time difference**
Average for 96 well plates	1 min 25 sec	5 min 10 sec	3 min 45 sec

Reproducibility of extractions

The A_{260}/A_{280} values obtained from the NanoDrop OneC Microvolume spectrophotometer for the DNA extractions ranged from 1.79 to 1.85 for automated and manually dispensed samples for both kits. Extraction-to-extraction variation is expected across all genomic and cell-free DNA samples based on inherent sample variability. The results from the Qubit dsDNA High Sensitivity Assay showed that there were differences in the DNA concentrations from samples using manual dispensing or the Multidrop Combi dispenser (Figure 2).

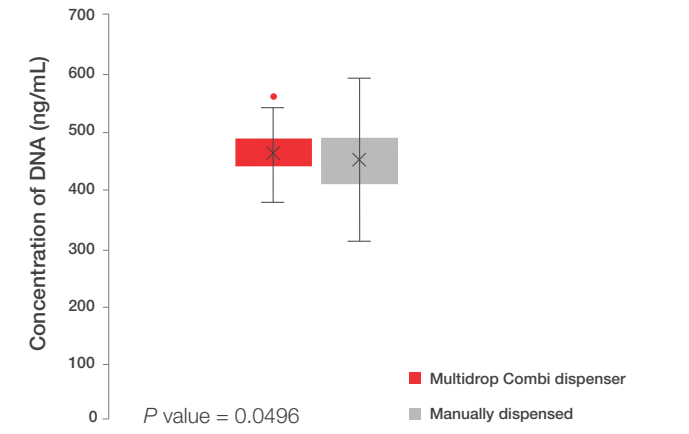
The plasma DNA concentrations in samples extracted with reagents dispensed using the Multidrop Combi dispenser (Figure 2A) ranged from 10.1 to 14.6 ng/mL in one plate, and using manually dispensed reagents, concentrations ranged from 8.72 to 13.1 ng/mL in one plate. The average concentration, standard deviation, and coefficient of variation (CV) of DNA for reagents dispensed with the Multidrop Combi dispenser were 12.3 ng/mL, 1.23, and 10%, respectively. The average concentration, standard deviation, and CV of DNA for the manually dispensed reagents were 10.5 ng/mL, 1.53, and 15%, respectively. The saliva DNA concentrations (Figure 2B) from 2 plates for reagents dispensed with the Multidrop Combi dispenser ranged from 378.0 to 560.0 ng/mL, and concentrations ranged from 310.0 to 590.0 ng/mL for manually dispensed reagents. The average concentration, standard deviation, and CV of DNA for reagents dispensed with the Multidrop Combi dispenser were 463.2 ng/mL, 34.42, and 7%, respectively. The average concentration, standard deviation, and CV of DNA for the manually dispensed reagents were 451.1 ng/mL, 59.6, and 13%, respectively. The Student's *t*-test calculations for the difference in the average concentration of DNA resulted in significant *P* values (< 0.05) (Figure 2). The *P* value was 0.0053 for the plasma samples and 0.0496 for the saliva samples (Figure 2B). Data analysis was performed using the 4200 TapeStation software.

A



Multidrop Combi dispenser			Manually dispensed		
Avg (ng/mL)	Std dev	CV (%)	Avg (ng/mL)	Std dev	CV (%)
12.3	1.23	10	10.5	1.53	15

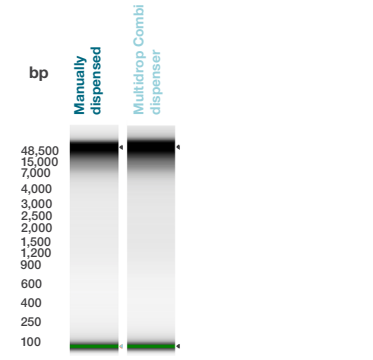
B



Multidrop Combi dispenser			Manually dispensed		
Avg (ng/mL)	Std dev	CV (%)	Avg (ng/mL)	Std dev	CV (%)
463.2	34.42	7	451.1	59.6	13

Figure 2. DNA concentrations for DNA extracted from (A) plasma and (B) saliva samples using reagents dispensed manually or by the Multidrop Combi reagent dispenser. Note the Student's *t*-test indicates that the average concentration of DNA is significantly different between dispensing methods for both types of samples.

A



B

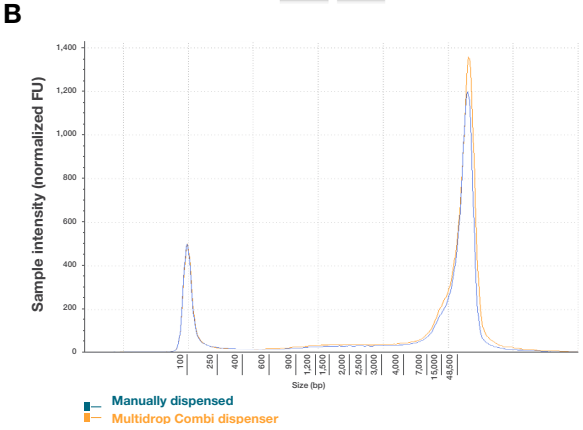


Figure 3. TapeStation software data on (A) e-gel analysis and (B) peak intensity of DNA from saliva samples extracted with reagents manually dispensed or dispensed by Multidrop Combi dispenser. The TapeStation software calculated a DIN of 8.4 for both dispensing methods. The peak sample intensity for both dispensing methods overlay one another, indicating a similar profile between extractions using manually dispensed reagents and those dispensed with the Multidrop Combi dispenser.

Figure 3 shows that the size of the DNA from saliva was above 48 kb, indicating that gDNA was efficiently extracted. The TapeStation software traces from saliva samples that were extracted with reagents using manual dispensing or automated dispensing overlay each other, indicating no difference between the dispensing methods. Also, the DIN was 8.4 for both dispensing methods, indicating that the gDNA is intact and shows no signs of shearing or degradation during extraction regardless of the dispensing method [1].

The results of qPCR from 3 separate plates using gDNA from saliva and the TaqMan RNase P Assay show that the threshold cycle (C_t) range is 25.319–25.919 with no amplification difference in the samples extracted with reagents using manual dispensing or the Multidrop Combi dispenser (Figure 4).

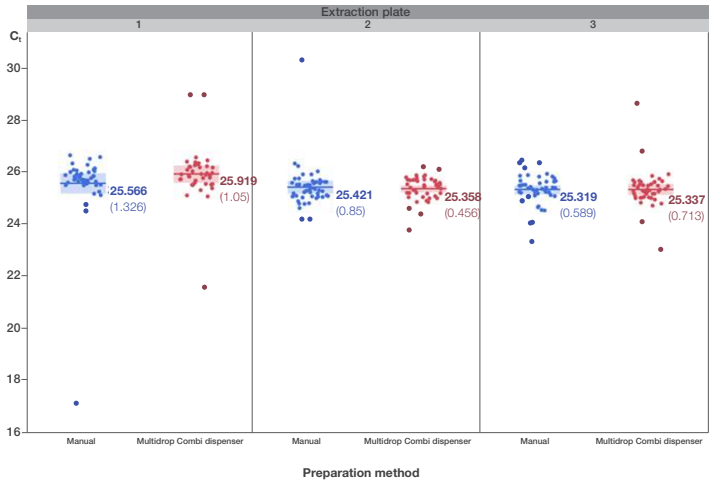


Figure 4. C_t values from the TaqMan RNase P Assay using gDNA from saliva samples treated with the SpeciMAX Stabilized kit and extracted with reagents dispensed manually or by the Multidrop Combi dispenser. Samples were extracted using the MagMAX DNA Ultra 2.0 kit.

Robustness of extractions

The 2100 Bioanalyzer system and the 4200 TapeStation system data show that the cfDNA peaks overlay one another, indicating a similar profile between extractions from plasma using manually dispensed reagents and automatically dispensed reagents with the Multidrop Combi dispenser using the MagMAX Cell-Free DNA Isolation Kit (Figure 5).

The primary cfDNA peak was visible in both traces in the 150–180 bp range, which is the typical size of cfDNA in plasma [2]. Additionally, comparison of the size profiles demonstrates that recovery levels of contaminating gDNA are not impacted by the reagent dispensing method.

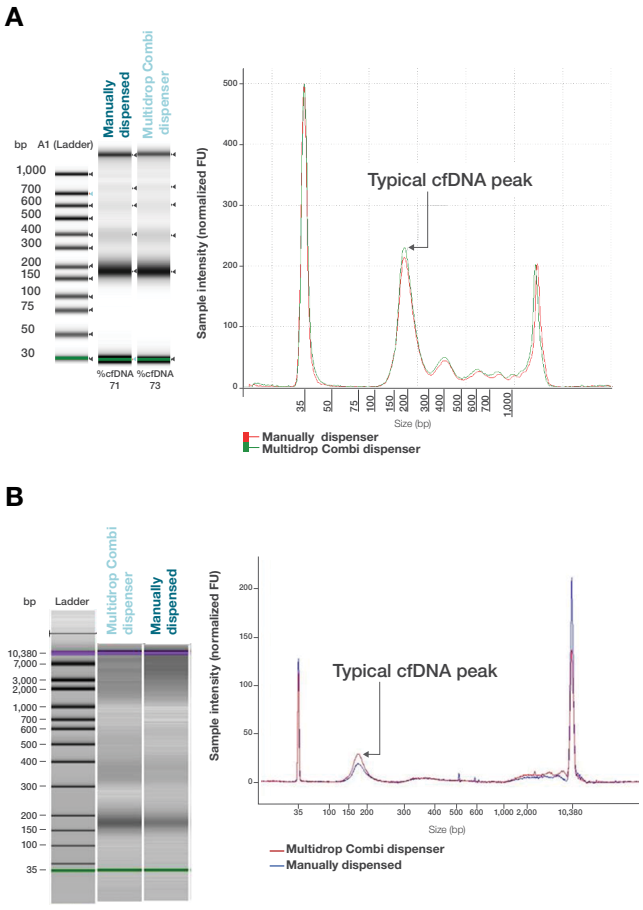


Figure 5. TapeStation software e-gel images (left) and 2100 Bioanalyzer system traces (right) of samples for which reagents were automatically dispensed using the Multidrop Combi dispenser or manually dispensed. Extractions from plasma samples took place in 2 separate labs—**(A)** Lab 1 and **(B)** Lab 2. Note that the peaks overlay one another in the traces from each lab, indicating a similar profile between extraction reagents that were manually dispensed or dispensed with the Multidrop Combi dispenser for both laboratories.

Conclusions

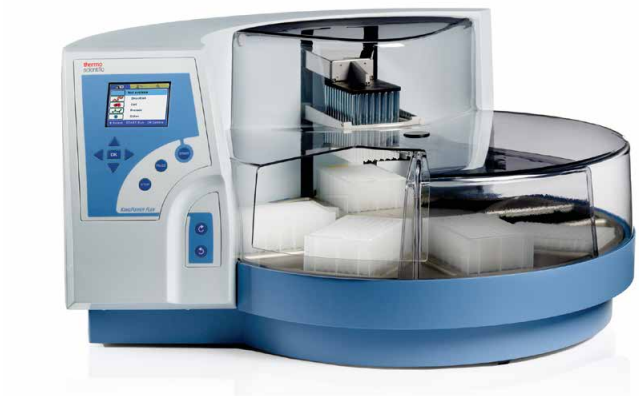
The purpose of this study was to evaluate the Multidrop Combi dispenser for dispensing of magnetic beads and reagents used in the MagMAX DNA extraction kits and determine its efficiency and reproducibility compared to manual dispensing methods. The results confirm that dispensing with the Multidrop Combi dispenser, when compared to manual dispensing, is more efficient, generates reproducible concentrations of DNA, and is repeatable when performed by different technicians in different laboratories.

In summary:

- The timing results show that dispensing with the Multidrop Combi dispenser is more efficient than manual dispensing, reducing the time by 62% to 73% when used for dispensing magnetic beads and reagents provided in MagMAX DNA isolation kits (Table 2).
- The A_{260}/A_{280} ratios for the DNA extractions were between 1.79 and 1.85 for both dispensing methods, indicating that the samples are clean and equally free from carry-over contaminants.
- The DNA concentrations show that both plasma and saliva samples for which magnetic beads and reagents were dispensed with the Multidrop Combi dispenser had less variation compared to those that were manually dispensed (Figure 2).
- The DIN of 8.4 for both dispensing methods as well as the overlapping peaks in the traces provide evidence that gDNA and cfDNA extracted using beads and reagents provided in the MagMAX kit and dispensed by Multidrop Combi dispensers are high in quality and similar to DNA extracted using reagents manually dispensed (Figure 3).
- The qPCR results demonstrated the endogenous RNase P gene is equivalently detected in saliva samples treated with the SpeciMAX Stabilized kit and with the MagMAX DNA Multi-Sample Ultra 2.0 kit using reagents dispensed either manually or automatically with the Multidrop Combi dispenser (Figure 4).
- Extraction of cfDNA from plasma using reagents dispensed by the Multidrop Combi dispenser is repeatable and therefore robust, as demonstrated by two different labs and technicians (Figure 5).

References

1. Padmanaban A (2015) **DNA integrity number (DIN) for the assessment of genomic DNA samples in real-time quantitative PCR (qPCR) experiments**. *Agilent Technologies*, publication number 5991-6368EN.
2. Volik S, Alcaide M et al. (2016) **Cell-free DNA (cfDNA): clinical significance and utility in cancer shaped by emerging technologies**. *Mol Cancer Res* 14(10):898–908.



Learn more at **thermofisher.com/multidrop**

thermo scientific

COL26431 0622