APPLICATION NOTE

KingFisher Apex instrument

KingFisher Apex instrument with dual magnet heads and upgraded cooling, heating, and elution features

Optimizing process efficiency and sample extraction

Key findings

- The addition of the option to switch between two magnet heads on the Thermo Scientific™ KingFisher™ Apex instrument allows running multiple extractions sequentially with various kits and methods without interruption, to swap out magnet heads. This saves time and resources for labs processing various sample types within a short time period.
- Process efficiency and sample quality are improved with the cooling and heating features on the upgraded KingFisher Apex instrument, as well as the ability to elute into storage tubes. Samples maintain a controlled environment during and after extraction, which can maintain sample quality and integrity. Eluting into storage tubes allows for easier, more efficient, and streamlined long-term storage of DNA and RNA samples, and cell elution from bead-based extraction enables long-term biobanking and downstream usability.
- Instrument decontamination is enabled with the addition of the UV light feature on the KingFisher Apex instrument.
- For RNA, DNA, and cell extraction, the KingFisher Apex instrument is comparable to its predecessor, the Thermo Scientific™ KingFisher™ Flex instrument, in yield, size, and purity.
- Barcoded and optimized plastics have been developed for improvements to workflows.

Introduction

Magnetic bead–based separation techniques have been a cornerstone for RNA, DNA, and cell extraction. The Thermo Scientific™ KingFisher™ instrument line, paired with various magnetic particle–based kits, utilizes these techniques to help achieve clean and high-quality extraction with consistent results. The KingFisher Flex instrument has been utilized with various Applied Biosystems™ MagMAX™ extraction kits to yield high-throughput extractions in various global diagnostic and research labs. Most recently, the KingFisher Flex instrument has been used with the Applied Biosystems™ MagMAX™ Viral/Pathogen II kit to extract SARS-CoV-2 viral RNA from nasopharyngeal swabs in viral transport media (VTM) and saliva samples with extremely high throughput during the recent global crisis of SARS-CoV-2 infections.

The KingFisher Apex instrument has been built upon the features of the KingFisher Flex instrument while continuing to provide the same high-quality results expected from magnetic particle–based high-throughput technologies. In addition to adopting cloud-based technologies and including improvements to the user interface with a touchscreen, the KingFisher Apex instrument has added features such as sample cooling, an option to switch between two magnet heads, the ability to elute into tubes, and UV light for decontamination. To demonstrate the features listed above, various RNA, DNA, and cell extractions were performed using a variety of sample types, including blood, biofluids, buffy coat, buccal swabs, plasma, and tissues, with the Applied Biosystems™ MagMAX™ extraction kits on the KingFisher Apex and KingFisher Flex instruments. Extraction efficiency and integrity, yield, and purity of nucleic acid were analyzed and compared for both instruments.

ThermoFisher SCIENTIFIC

Bead-based extractions with MagMAX kits

Materials and methods

To demonstrate that the KingFisher Apex instrument is comparable to the KingFisher Flex instrument, five different Applied Biosystems™ MagMAX™ kits were used to extract nucleic acid using various workflows, sample types, and inputs on both the KingFisher Apex and KingFisher Flex instruments (Figure 1).

Figure 1. MagMAX kits used with the KingFisher Flex and KingFisher Apex instruments. The Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kit, MagMAX™ *mir*Vana™ Total RNA Isolation Kit, MagMAX™ Cell-Free DNA Isolation Kit, MagMAX™ Cell-Free TNA Isolation Kit, and MagMAX™ FFPE DNA/RNA Ultra Kit were all used on both the KingFisher Apex and KingFisher Flex instruments.

All experiments on the KingFisher Apex instrument were performed in parallel on the KingFisher Flex instrument. Deep-well plates (with 96 or 24 wells) were used in addition to the Combi tip comb to evaluate the functionalities of the KingFisher Apex instrument for this study. Formalin-fixed, paraffin-embedded (FFPE) skin, lung, colon, and breast tissue samples were processed with the MagMAX FFPE DNA/RNA kit using both standard- and large-volume protocols. Human whole blood as well as buffy coat samples were used with the MagMAX DNA Multi-Sample Ultra 2.0 Kit using various input volumes of 200 µL, 400 µL, 1 mL, and 2 mL. Whole blood was also processed with the MagMAX *mir*Vana kit, using the biofluids protocol. The cells and tissues protocol was used for both HeLa and Huh7 cells at various inputs. Plasma samples were processed using the MagMAX Cell-Free DNA Isolation Kit and the MagMAX Cell-Free TNA Isolation Kit, using the 2 mL and 4 mL protocols. RNA and DNA yields were analyzed with the Invitrogen™ Qubit™ 4.0 Fluorometer using the Invitrogen™ Qubit™ RNA HS Assay and the Invitrogen™ Qubit™ dsDNA HS Assay, respectively. The Thermo Scientific™ NanoDrop™ 8000 Spectrophotometer was used to analyze nucleic acid sample purity by looking at A_{260}/A_{230} and A_{260}/A_{280} ratios. Furthermore, to evaluate quality, the Agilent Bioanalyzer™ 2100 system was used as necessary to determine peak sizes of genomic DNA (gDNA) and cfDNA as appropriate for individual extraction methods. Depending on the protocol used, qPCR was run to detect mRNA, miRNA, or DNA on the Applied Biosystems™ QuantStudio™ 12K Flex instrument with appropriate reaction reagents.

Results and discussion

MagMAX FFPE DNA/RNA Ultra Kit

FFPE breast, colon, and skin tumor samples were used with the standard-volume protocol with the MagMAX FFPE DNA/RNA Ultra Kit. FFPE skin, lung, and breast tumor samples were used for extraction utlizing the high-volume protocol. Figure 2 details total RNA and DNA yields from both KingFisher Flex and Apex samples showing equivalency within standard deviations for all three samples using their respective extraction protocols.

A DNA qPCR target (GAPDH_g1) was used to measure DNA yields on the QuantStudio 12K Flex instrument. mRNA targets (GAPDH_m1 and beta-actin ACTB_m1) were analyzed in the same fashion on both the KingFisher Flex and Apex instruments.

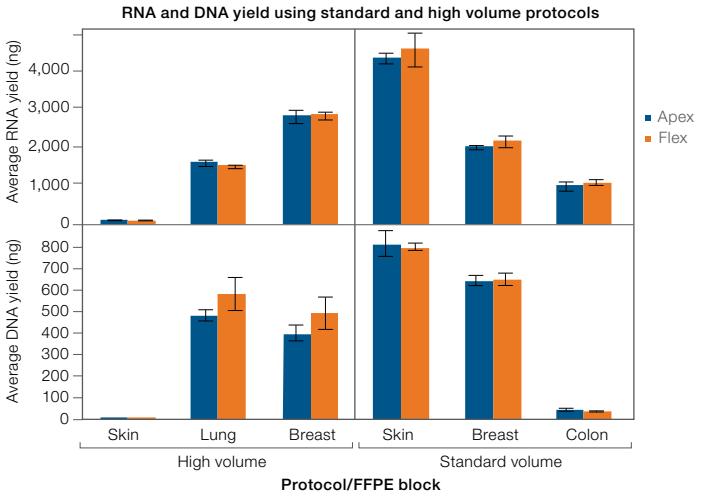

Figure 2. RNA and DNA yield using standard- and high-volume protocols using the KingFisher Flex and Apex instruments. FFPE breast, skin, lung, and colon samples were used for extraction utilizing the standard-volume protocol while FFPE breast, lung, and skin samples were used for extraction utilizing the high-volume protocol using the MagMAX FFPE DNA/RNA Ultra Kit. DNA and RNA yields were measured with Qubit assays and their respective protocols.

Figure 3 demonstrates that extractions from FFPE skin, colon, and breast tumor samples utilizing the standard protocol for the MagMAX FFPE DNA/RNA Ultra Kit on the KingFisher Flex and Apex instruments yield C_t values within a range of 1 cycle, indicating extraction equivalence between the two instruments.

Figure 4 demonstrates qPCR data for DNA (GAPDH_g1) as well as mRNA targets (GAPDH_m1 and ACTB_m1) for the high-volume protocol with skin, lung, and breast tumor samples. DNA and mRNA yields from the KingFisher Flex and Apex instruments were found to be equivalent.

To determine equivalency of RNA size profiles between the two instruments, the Bioanalyzer instrument with the Agilent™ High Sensitivity (HS) DNA Chip was used. Figures 5 and 6 show RNA size profile equivalency between matched FFPE tumor samples extracted on the KingFisher Flex and Apex instruments utilizing the standard- and high-volume protocols, respectively.

miRNA-specific qPCR targets, let7e and mir16, were targeted for FFPE tumor samples extracted with both standard- and high-volume protocols. The Applied Biosystems™ TaqMan® microRNA Reverse Transcription Kit was utilized post-extraction and run with Applied Biosystems™ TaqMan® Universal Master Mix on the QuantStudio 12K Flex instrument. Figure 7 shows C_t values between the KingFisher Flex and Apex instruments are all within 1 cycle across both let7e and mir-16 assays for samples extracted with standard- and high-volume protocols.

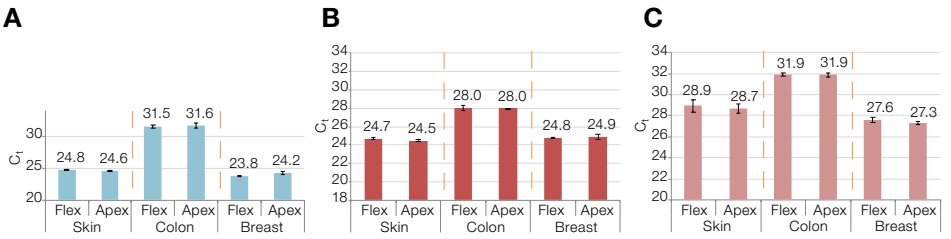

Figure 3. qPCR data of DNA and mRNA targets from FFPE tumor samples extracted using the standard-volume protocol. C_t for **(A)** GAPDH DNA, **(B)** GAPDH_m1 RNA, and **(C)** ACTB_m1 RNA from FFPE breast, colon, and skin tumor samples extracted using the standard-volume protocol on the KingFisher Flex and Apex instruments.

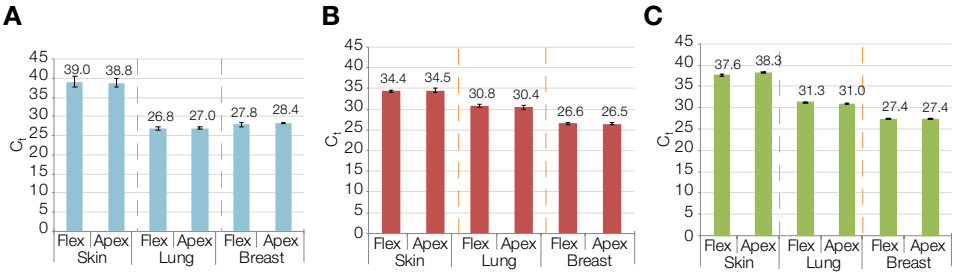

Figure 4. qPCR data of DNA and mRNA targets from FFPE tumor samples extracted using the high-volume protocol. C_t for **(A)** GAPDH DNA, **(B)** GAPDH_m1 RNA, and **(C)** ACTB_m1 RNA from FFPE skin, lung, and breast tumor samples extracted using the high-volume protocol on the KingFisher Flex and Apex instruments.

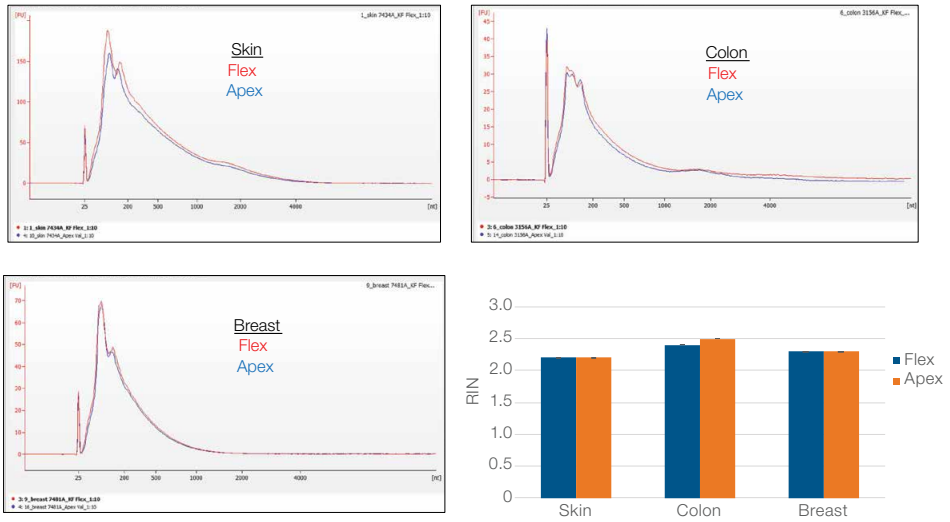

Figure 5. RNA size profiles and RIN values for samples extracted using the standard-volume protocol. Results obtained on the Bioanalyzer system indicate matching RNA size profiles for extractions using the KingFisher Flex and Apex instruments with the standard-volume protocol.

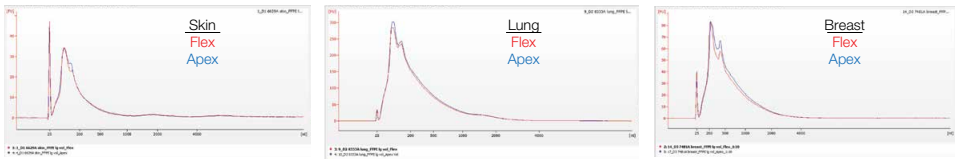

Figure 6. RNA size profiles and RIN values for samples extracted using the high-volume protocol. Results obtained on the Bioanalyzer system indicate matching RNA size profiles for extractions using the KingFisher Flex and Apex instruments with the high-volume protocol.

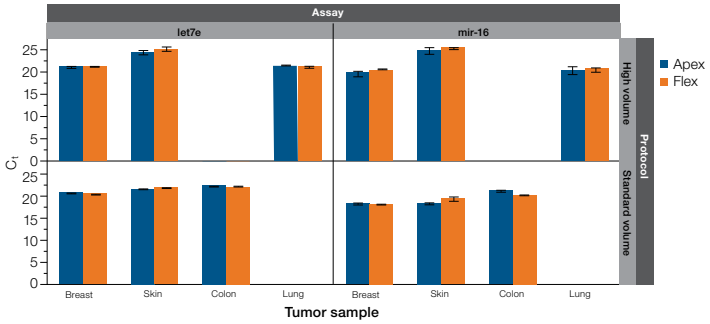

Figure 7. qPCR results for miRNA targets with high- (top) and standard- (bottom) volume protocols. FFPE breast, skin, and colon samples were extracted with the standard-volume protocol, while FFPE breast, skin, and lung samples were extracted with the high-volume protocol, on both the KingFisher Flex and Apex instruments. In both protocols, cDNA templates were synthesized with the TaqMan microRNA RT kit, and qPCR was run to determine miRNA in samples with let7e and mir-16 as the qPCR targets.

MagMAX Multi-Sample Ultra 2.0 Isolation Kit

Blood, buffy coat, and buccal swabs were extracted on both the KingFisher Apex and Flex instruments with the MagMAX Multi-Sample Ultra 2.0 Isolation Kit. Whole blood was extracted from two donor samples using protocols for 200 µL, 400 µL, 1 mL, and 2 mL sample volumes, while determining the DNA yield and purity ratios for each volume. Samples from three donor samples were used for the buffy coat protocol and were extracted on both the KingFisher Flex and the KingFisher Apex instruments. The eluate was evaluated with the Qubit 4.0 Fluorometer to determine DNA yield and the NanoDrop 8000 Spectrophotometer to determine purity ratios. The buccal swab protocol was utilized to extract samples from six donors, using both the KingFisher Flex and Apex instruments with one swab per extraction. DNA yield was determined on the Qubit 4.0 Fluorometer and purity ratios were determined on the NanoDrop 8000 Spectrophotometer. Figure 8 details the yield across all protocols (blood at various inputs, buffy coat, and buccal swabs). Total DNA yield in the elution is equivalent for samples extracted from both the KingFisher Flex and Apex instruments.

The purity of all extractions was determined using NanoDrop dsDNA modules. Matched samples on the KingFisher Flex and Apex instruments showed equivalent absorbance ratios (A_{260}/A_{280} and A_{260}/A_{230}) for protocols with blood sample inputs of 200 µL, 400 µL, 1 mL, and 2 mL; buffy coat samples from three donors; and buccal swabs (Figures 9 and 10). This indicates that the nucleic acid from samples processed on the KingFisher Apex instrument is equivalent to samples processed on the KingFisher Flex instrument. Figure 10 also indicates that A_{260}/A_{230} ratios for buccal swabs are lower overall, which is possibly due to variations in sample types as opposed to instrument platform as data from both the KingFisher Flex and Apex instruments are within appropriate standard deviations between donor samples.

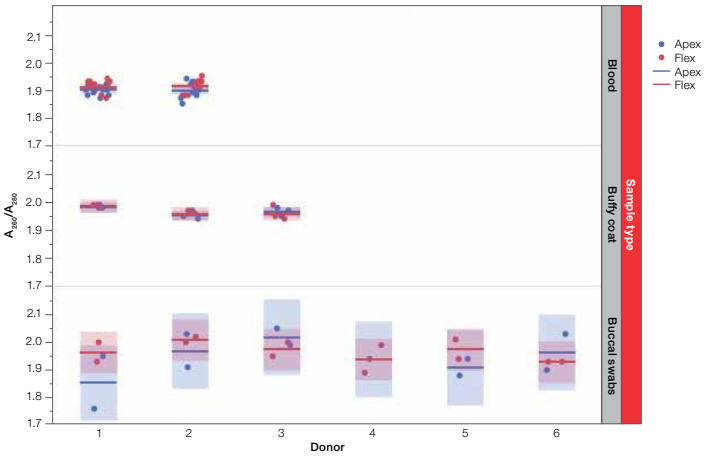

Figure 9. Purity ratios on NanoDrop 8000 Spectrophotometer across all protocols. A_{260}/A_{280} ratios across protocols for blood samples (200 µL, 400 µL, 1 mL, and 2 mL inputs), buffy coat, and buccal swabs.

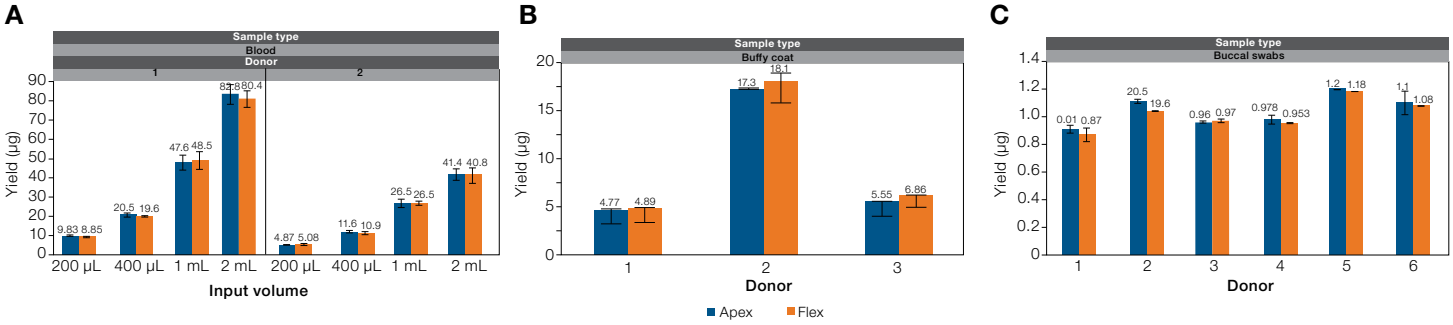

Figure 8. Total yield from (A) whole blood, (B) buffy coat, and (C) buccal swabs extracted with the MagMAX Multi-Sample Ultra 2.0 Kit on the KingFisher Flex and Apex instruments at various input volumes.

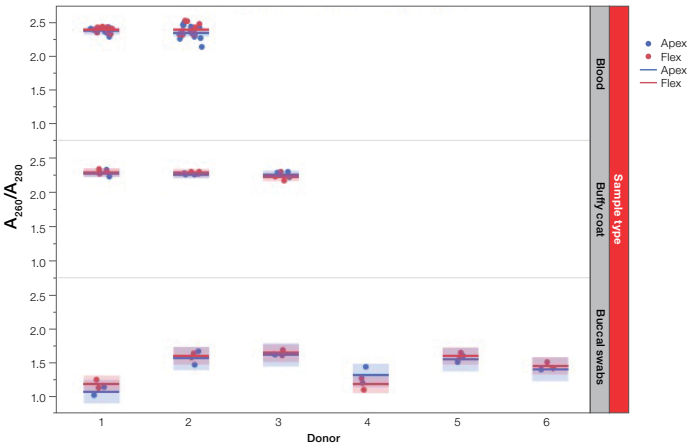

Figure 10. Purity ratios on NanoDrop 8000 Spectrophotometer across all protocols. A_{260}/A_{230} purity ratios across protocols for blood samples (200 µL, 400 µL, 1 mL, and 2 mL inputs), buffy coat, and buccal swabs.

MagMAX *mir*Vana Total RNA Isolation Kit

Extractions on 2.5 x 10^5, 5 x 10^5, and 10^6 HeLa and Huh7 cells were performed using the MagMAX *mir*Vana Total RNA Isolation Kit on both the KingFisher Apex and Flex instruments using the cells and tissues protocol. Similarly, the biofluids protocol was used to extract whole blood from two donor samples. For all extractions, RNA yield was measured with the Qubit RNA HS Assay Kit and qPCR was performed to detect mRNA and miRNA targets. Integrity and RNA size were measured by RIN value and peak profiles on the Bioanalyzer instrument. Figure 11 indicates the total RNA yield using the Qubit RNA HS Assay Kit for HeLa and Huh7 cells extracted with the cells and tissues protocol, as well as for whole blood extracted with the biofluids protocol. Samples extracted with the KingFisher Apex instrument had similar or better yields than samples extracted with the KingFisher Flex instrument across all platforms.

Invitrogen™ SuperScript™ VILO™ Master Mix was used to perform reverse-transcription (RT) reactions on nucleic acid extracted from HeLa and Huh7 cells as well as whole blood. After the RT reaction, a qPCR reaction with Applied Biosystems™ TaqMan® Universal Master Mix II, with no UNG, and TaqMan® primers for mRNA assays targeting beta-actin (m1_ACTB) and GAPDH (m1_GAPDH) was run. Figure 12 shows C_t equivalency within a range of 1 amplification cycle between both instrument platforms across both mRNA targets for 2.5 x 10^5, 5 x 10^5, and 10^6 HeLa and Huh7 cell inputs as well as whole blood extractions. Figure 12 also shows that ACTB targets did not amplify earlier or decrease, with increasing inputs across HeLa and Huh7 cells as expected. This observation can be seen with both the KingFisher Flex and Apex instruments and is not an instrument-related issue.

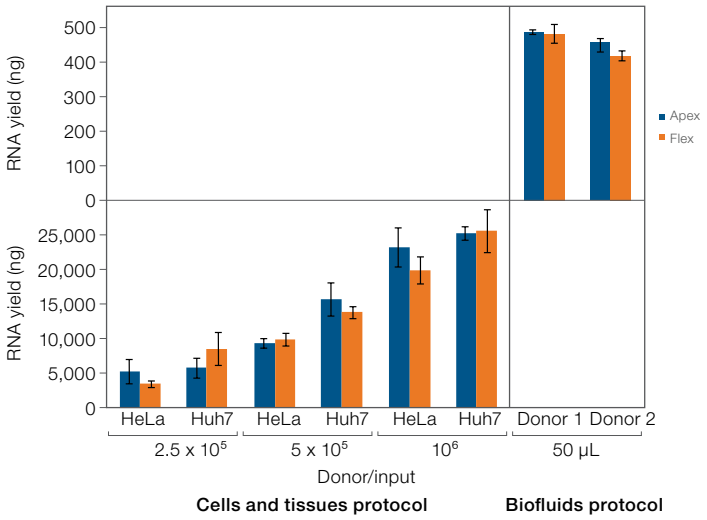

Figure 11. Total RNA yield (ng) from the extracted samples, using the cells and tissues and biofluids protocols. HeLa and Huh7 cells were processed at 2.5 x 10^5, 5 x 10^5, and 10^6 cells per extraction following the cells and tissues protocol, and 50 µL of whole blood was processed from two donor samples following the biofluids protocol, using the MagMAX *mir*Vana Total RNA Isolation Kit.

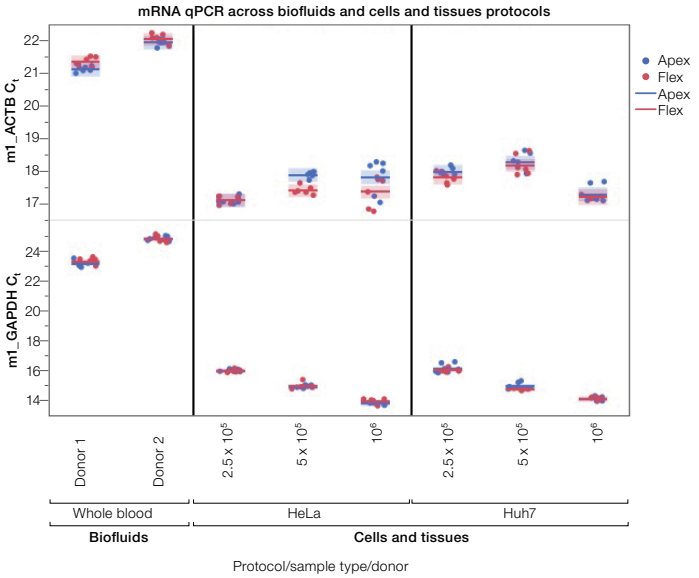

Figure 12. qPCR of mRNA targets (m1_ACTB and m1_GAPDH) from nucleic acid extracted with the cells and tissues and biofluids protocols. qPCR of mRNA targets m1_ACTB and m1_GAPDH with extracts of 2.5 x 10^5, 5 x 10^5, and 10^6 HeLa and Huh7 cells, as well as 50 µL of whole blood, extracted across two donor samples on the KingFisher Apex and Flex instruments.

Using TaqMan primers and reagents, an RT reaction was conducted to detect miRNA targets mir-24 and mir-16. Following the RT reaction, a qPCR reaction was set up with TaqMan Universal Master Mix II, with no UNG, to detect mir-24 and mir-16 targets in nucleic acid from HeLa and Huh7 cells as well as from whole blood using the MagMAX *mir*Vana Total RNA Isolation Kit on both the KingFisher Apex and Flex instruments. Figure 13 indicates that C_t values for miRNA qPCR targets mir-16 and mir-24 are equivalent on matched samples across the KingFisher Apex and Flex instruments. The KingFisher Apex instrument showed lower C_t values than extractions from the KingFisher Flex instrument across both miRNA targets with the higher input (10^6 cells) of HeLa and Huh7 cells.

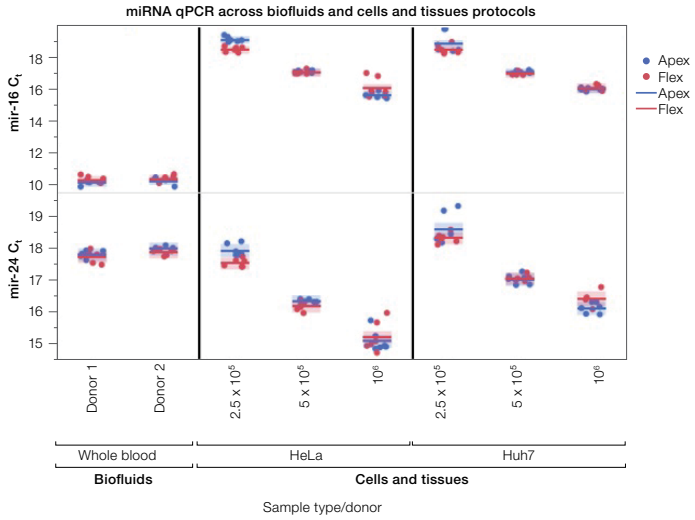

Figure 13. qPCR of miRNA targets (mir-16 and mir-24) from nucleic acid extracted with the cells and tissues and biofluids protocols. qPCR of miRNA targets mir-16 and mir-24 synthesized from HeLa and Huh7 cell extracts at 2.5 x 10^5, 5 x 10^5, and 10^6 inputs, as well as from extracts of 50 µL of whole blood from two donor samples, on the KingFisher Apex and Flex instruments.

The Bioanalyzer system was utilized with the Agilent™ RNA 6000 Nano Kit to evaluate peak profile and RIN values for samples extracted from HeLa and Huh7 cells using the cells and tissues protocol. Figure 14 indicates that the nucleic acid extracted on the KingFisher Apex and KingFisher Flex instruments yielded RNA of equivalent sizes. The RIN (RNA integrity number) values were equivalent as well.

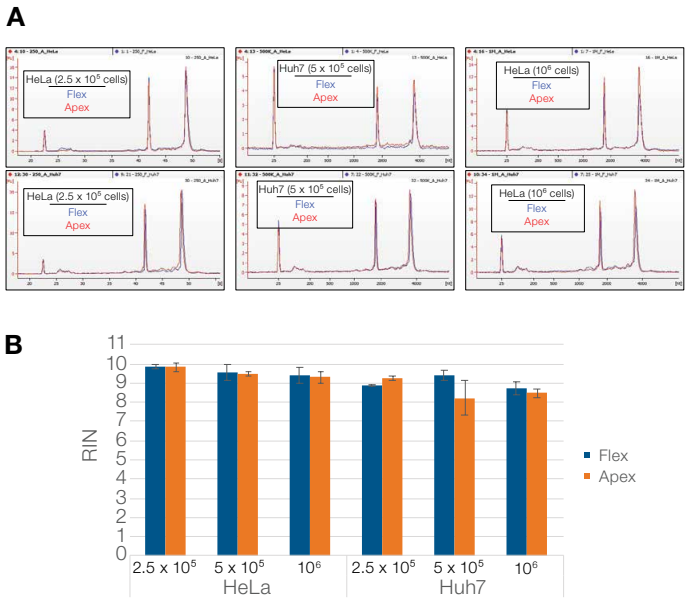

Figure 14. Determination of RNA size and integrity on the Bioanalyzer system. (A) HeLa and Huh7 cells extracted at various inputs with the cells and tissues protocol were run on the Bioanalyzer system utilizing the RNA 6000 Nano Kit. **(B)** RIN values were reported for sample integrity.

MagMAX Cell-Free DNA Isolation Kit

Cell-free plasma was extracted using the 2 mL and 4 mL protocols for the MagMAX Cell-Free DNA Isolation Kit on both the KingFisher Apex and KingFisher Flex instruments across three donor samples. Figure 15 details cell-free DNA yield and size equivalency between the KingFisher Flex and the KingFisher Apex instruments. Fragment sizes were assessed on the Bioanalyzer system with the Agilent™ HS DNA Assay. Percentages of cfDNA relative to gDNA extracted were evaluated to be equivalent or higher with the KingFisher Apex instrument than in matched samples extracted using the KingFisher Flex instrument.

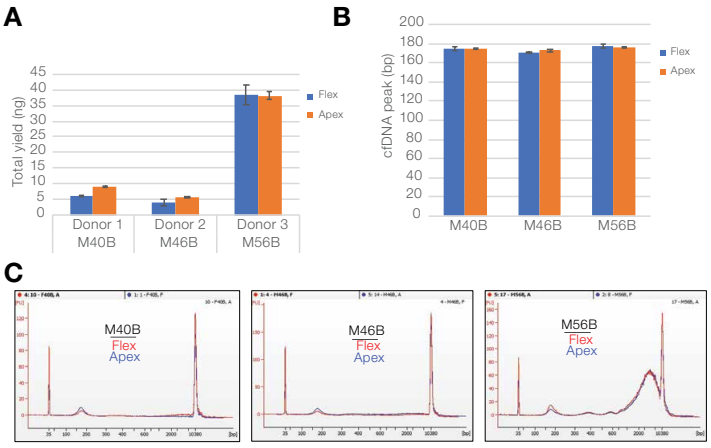

Figure 15. Yield and size comparison with 2 mL protocol on the KingFisher Flex and Apex Instruments using three donor samples. (A) cfDNA yield was analyzed with the Qubit dsDNA HS Assay for three donor samples. **(B)** cfDNA peak size (bp) and **(C)** profiles were analyzed with the Agilent HS DNA Assay.

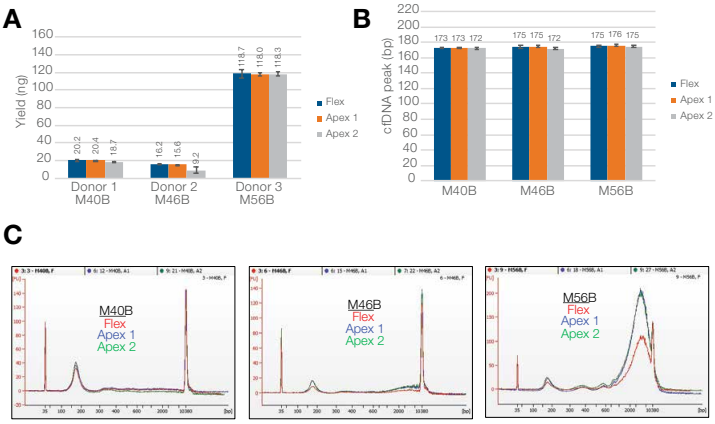

Figure 16. Yield and size comparison with 4 mL protocol for three donor samples on the KingFisher Flex and two KingFisher Apex instruments. (A) cfDNA yield was analyzed with the Qubit dsDNA HS Assay for three donor samples. **(B)** cfDNA peak size and **(C)** profiles were analyzed with the Agilent HS DNA Assay.

The 4 mL protocol was utilized across two KingFisher Apex instruments to determine instrument-to-instrument variability. Yield and size of cfDNA was analyzed with the Qubit HS dsDNA Assay along with the Agilent HS DNA Assay. Figure 16 shows that there is equivalency between all three instruments (one KingFisher Flex and two KingFisher Apex systems). For the second KingFisher Apex instrument ("Apex 2") the sample from one donor had a lower yield and a larger variation. This is likely related to plasma quality and has been ruled out as being related to instrument performance (Figure 16).

MagMAX Cell-Free Total Nucleic Acid Isolation Kit

Cell-free plasma was extracted with the 2 mL and 4 mL protocol using both the KingFisher Apex and Flex instruments with the MagMAX Cell-free Total Nucleic Acid Isolation Kit. Figure 17 indicates yield and size equivalency between both the KingFisher Flex and Apex instruments by analyzing cfDNA peak size calculated by the Bioanalyzer instrument using HS DNA Assay. Equivalency with percent cfDNA was observed between the two instrument platforms. It was noted that one donor sample, F58C, had a significant gDNA peak present in extractions using both instruments that overlapped with the upper marker, effectively preventing accurate quantification of cfDNA peaks, so this donor was omitted from the percent cfDNA analysis.

Like the 2 mL protocol, the 4 mL protocol underwent similar analysis for DNA peak size and percent cfDNA with the Qubit dsDNA HS Assay and Bioanalyzer instrument. Figure 18 shows that total cfDNA yields, as measured by the Qubit dsDNA HS Assay from samples extracted by the KingFisher Apex instrument, were equivalent to matched

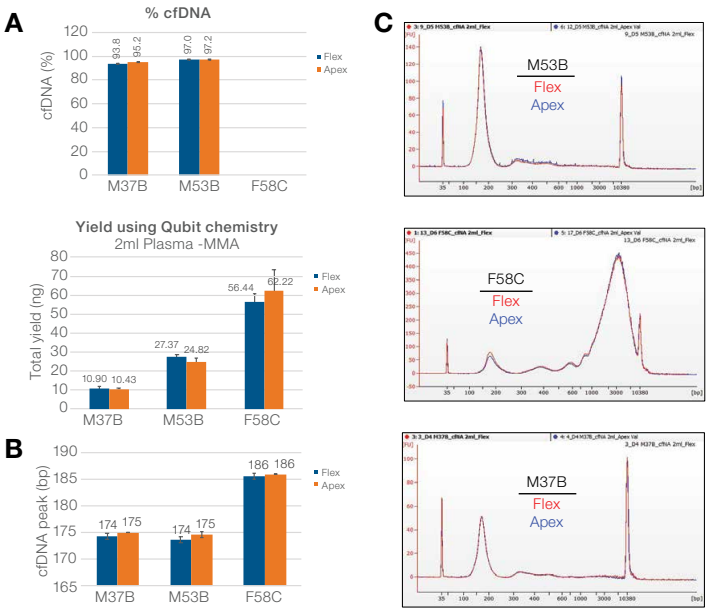

Figure 17. cfDNA yield and size comparison with 2 mL protocol on the KingFisher Flex and Apex instruments. (A) cfDNA yield was analyzed with the Qubit dsDNA HS Assay for three donor samples. **(B)** cfDNA peak size and **(C)** profiles were analyzed with the Agilent HS DNA Assay. High gDNA content was present in donor F58C, which obscured the upper markers so that cfDNA could not be quantified. This occurred for extractions using both the KingFisher Apex and Flex instruments, and was due to the quality of the donor samples and not the instrumentation.

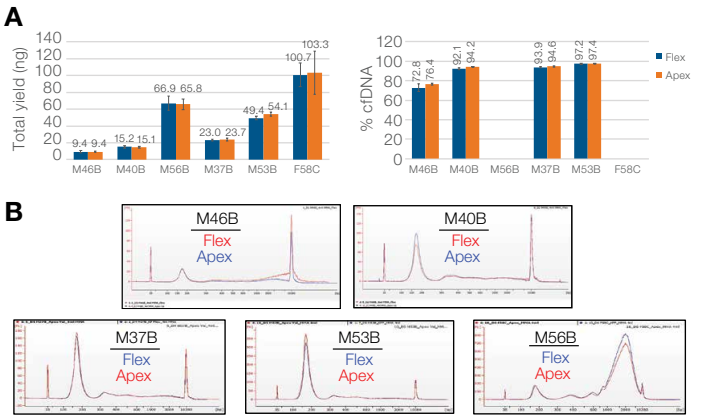

Figure 18. Yield and size comparison with 4 mL protocol on the KingFisher Flex and Apex instruments. (A) cfDNA yield was analyzed with the Qubit dsDNA HS Assay for 6 donor samples. High gDNA content was present in donors M56B and F58C, which obscured the upper markers so that cfDNA could not be quantified. This occurred for extractions using both the KingFisher Apex and Flex instruments, and was due to the quality of the donor samples and not the instrumentation. **(B)** cfDNA peak sizes (bp) and profiles were analyzed with the Agilent HS DNA Assay.

samples extracted on the KingFisher Flex instrument. Donor samples M56B and F58C had significant gDNA content, which caused high replicate variability and standard deviations and would not allow for percent cfDNA calculation, as the upper markers were obscured on the Bioanalyzer system. The cfDNA peak calculated by the Bioanalyzer instrument shows equivalency between the KingFisher Flex and Apex instruments.

Total cfRNA was analyzed with m1_GAPDH and m1_ACTB by qPCR using TaqMan Assays on the QuantStudio 12K Flex instrument. Figure 19 shows data from both 2 mL and 4 mL extraction protocols across m1_ACTB and m1_GAPDH targets. For the 2 mL protocol, both ACTB and GAPDH targets show equivalency between the KingFisher Flex and Apex Instruments. The 4 mL protocol shows equivalent C_t values between KingFisher Flex and Apex instruments except for donor sample M40B. C_t values for donor sample M40B were higher (amplified later) with the KingFisher Apex extractions and not within the standard deviation of the replicates. Three additional donor samples were added to the experiment on a second extraction run. For the three new donor samples, C_t values were equivalent between the KingFisher Flex and the KingFisher Apex instruments across both ACTB and GAPDH targets. This indicates an operator or sample error and is not due to instrumentation.

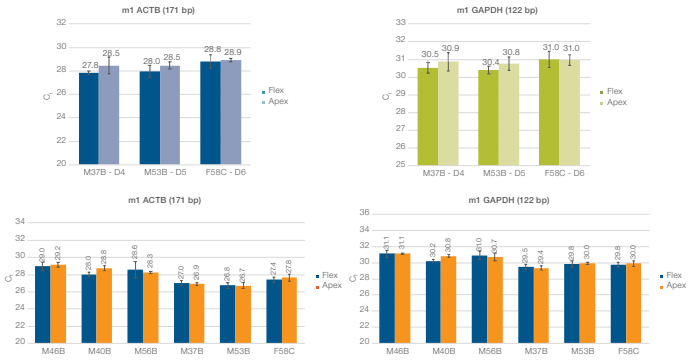

Figure 19. qPCR of *GAPDH* and *ACTB* targets from cell-free plasma samples extracted using 2 mL and 4 mL protocols. m1_ACTB and m1_GAPDH targets using 2 mL and 4 mL extraction protocols with the MagMAX Cell-Free Total Nucleic Acid Isolation Kit.

Elution into tubes

The MagMAX DNA Multi-Sample Ultra 2.0 Kit was used to demonstrate the ability to elute into tubes for whole blood samples utilizing the 2 mL protocol. For this experiment, elution into storage tubes (ST elution) was compared to elution into 24 deep-well plates by the Thermo Scientific™ KingFisher™ Apex 24 Combi tip on the KingFisher Apex instrument as well as 24 deep-well plates on the KingFisher Flex instrument. Figure 20 shows yield and purity results from using the Qubit HS DNA Assay as well as the NanoDrop 8000 Spectrophotometer to evaluate equivalency of dsDNA and ssDNA quality between matched samples. There are some variations between KingFisher Apex storage tube elution and the KingFisher Flex control, which is likely due to lower liquid temperature when using a 24-tube storage for elution compared to 24 deep-well plates. A_{260}/A_{280} and A_{260}/A_{230} absorbance ratios as well as DNA size was found to be equivalent between all three extraction platforms. This indicates that elution into storage tubes (STs) shows equivalent performance to elution into deep-well plates, which is useful for biobanking and long-term nucleic acid storage.

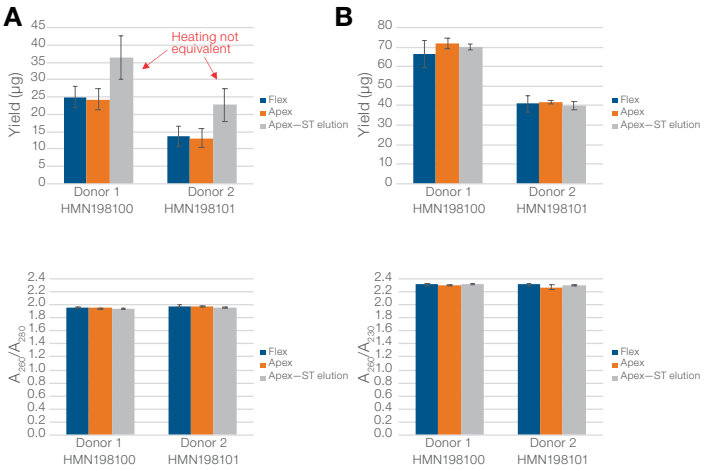

Figure 20. Yield and purity of DNA extracted from blood samples with 2 mL protocol extraction using MagMAX DNA Multi-Sample Ultra 2.0 and eluted into storage tubes (ST) vs. deep-well plates. (A) Double-stranded DNA yield determined using the Qubit HS DNA Assay; **(B)** ssDNA yield determined on a NanoDrop 8000 Spectrophotometer; **(C)** A_{260}/A_{280} and **(D)** A_{260}/A_{230} across KingFisher Flex and Apex instruments eluted into both 24 deep-well plate format as well as storage tubes.

Cooling feature addition

In addition to the efficient heating capabilities of the KingFisher Flex instrument, the KingFisher Apex has included a cooling block. This is beneficial for sample integrity post-extraction as well as protein extractions. To demonstrate this capability, a Digi-Sense™ Thermocouple thermometer was utilized with Type-K Digital Thermometer Probes and placed into two corners and two center wells of a 24 deep-well plate filled with 500 µL of water and repeated with a 96 deep-well plate filled with 100 µL of water (Figure 21). The thermal profile was read after 16 hours.

After 16 hours on the KingFisher Apex instrument, the Digi-Sense Thermometer was analyzed. Figure 22 shows that all samples were able to reach temperature below 8˚C within the allotted time. After 16 hours, condensation was observed within the cooling block. This condensation was localized within one side of the cooling block with the instrument being level. This observation indicates that cooling temperatures should be set higher than 4˚C, especially for longer incubation times.

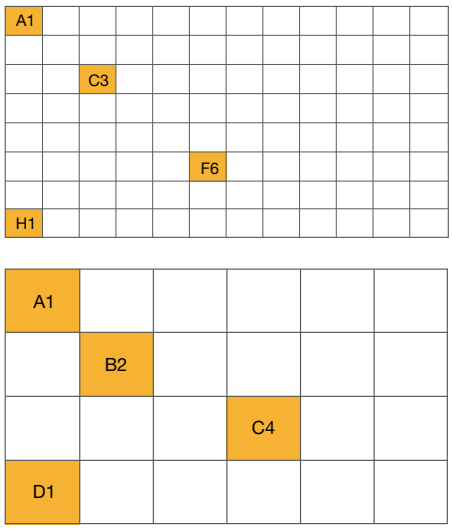

Figure 21. Thermocouple probe placement in 96 deep-well and 24 deep-well plates on the KingFisher Apex instrument to examine cooling and heating profiles.

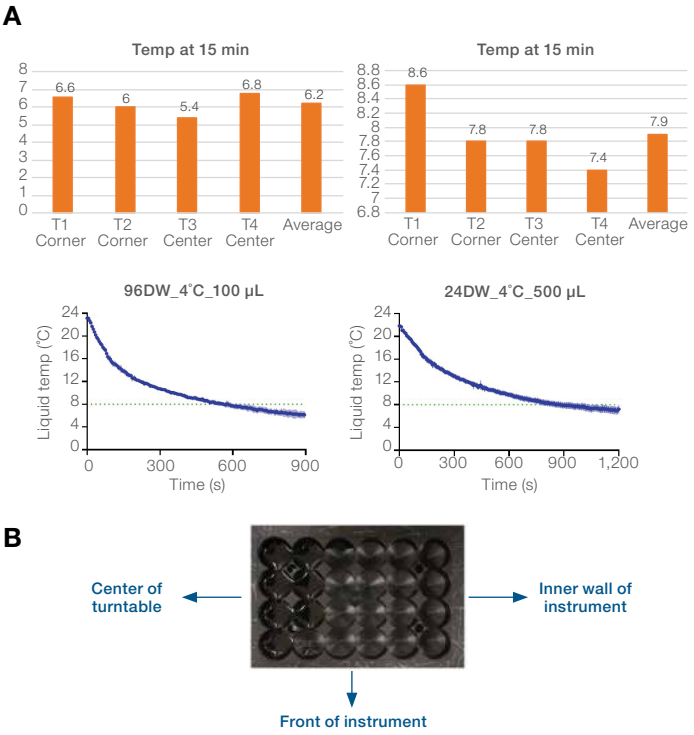

Figure 22. Cooling profile and plate condensation over time.
(A) Cooling profile of 96 deep-well and 24 deep-well plates set to 4˚C on the KingFisher Apex instrument at water volumes of 100 µL and 500 µL. **(B)** Data showing condensation within the cooling block after 16 hours.

Decontamination with the UV light

A UV light feature has been added to the KingFisher Apex instrument for decontamination applications and needs. Decontamination efficiency of the KingFisher Apex instrument was evaluated using GFP-expressing *E. coli* sourced from ATCC. The 30 min decontamination protocol was run on the KingFisher Apex instrument, and the Grant bio DNA/RNA UVC/T-M-AR cabinet was used as a reference for the UV light within the KingFisher Apex instrument.

For this test, two cultures of *E. coli* inoculated into 5 mL of LB broth containing 0.1 mg/mL ampicillin were incubated overnight at 30˚C with shaking at 200 rpm. After ~16 hours, a 1 mL sample was pulled from the culture and diluted 1:10, and then 200 µL of this sample was transferred to a 96-well plate where optical density was measured at 600 nm with a Thermo Scientific™ Multiskan™ SkyHigh Microplate Spectrophotometer.

The overnight culture was used to start a new *E. coli* GFP culture at ~OD_{600} of 0.1. The culture was incubated at 37˚C with shaking at 150 rpm, until the OD_{600} was 0.58. A 10-fold dilution series down to 10^{-7} was made, and plated onto replicate plates for each testing parameter (Apex UV, Grant bio UV, and no UV exposure); 10 plates were placed into the KingFisher Apex instrument without lids (Figure 23), and 10 plates at the same concentrations were placed into the Grant bio cabinet without lids, and UV lamps were switched on for 30 min. In addition, 5 control plates were left out and not exposed to UV light. After the 30 min exposure to UV light, the lids were returned to the LB plates and they were incubated at 37˚C overnight. After 16 hours, the colonies were counted. No colonies were found on the KingFisher Apex plates nor on the Grant bio reference system plates, whereas the plates left out of the systems showed significant growth, ~200 colonies/plate, reflecting an average of 2.1 x 10^9 CFU/mL.

Figure 23. Example of LB plate layout within the KingFisher Apex instrument for 30 min decontamination protocol.

Conclusion

Evaluation of the bead-based extractions kits with the MagMAX DNA Multi-Sample Ultra 2.0 Kit, MagMAX Cell-Free DNA Isolation Kit, MagMAX Cell-Free Total Nucleic Acid Isolation Kit, MagMAX *mir*Vana Total RNA Isolation Kit, and MagMAX FFPE DNA/RNA Ultra Kit showed equivalency between the KingFisher Flex and Apex instruments in yield and purity, as demonstrated by data from the Qubit and Bioanalyzer instruments, and absorbance data with the NanoDrop 8000 Spectrophotometer. It also showed equivalency between the two instrument platforms in retention of DNA, mRNA, and miRNA as shown with the RT-qPCR and qPCR results. The ability to elute the sample extracts into tubes for biobanking purposes did not vary between the KingFisher Flex and Apex instruments, which suggests suitable elution for both tubes and plate formats. The new cooling feature is an efficient way to maintain nucleic acid integrity post-extraction by keeping them cool until you can seal the elution plate. Cooling temperatures should be kept above 4˚C for extended periods of time. The KingFisher Apex instrument showed no growth on plates exposed to the internal UV lights for 30 minutes, which supports the successful decontamination feature of the system. The availability of the UV lights for decontamination within the instruments is extremely beneficial when working with high-titer material for extractions.

Ordering information

Product	Cat. No.
Instruments	
KingFisher Apex instrument with 96 PCR head	5400910
KingFisher Apex instrument with 96 Combi head	5400920
KingFisher Apex instrument with 96 deep-well head	5400930
KingFisher Apex instrument with 24 Combi head	5400940
KingFisher Flex Purification System, with 96 PCR head	5400610
KingFisher Flex Purification System, with 96 KF head	5400620
KingFisher Flex Purification System, with 24 deep-well head	5400640
KingFisher Flex Purification System, KingFisher with 96 deep-well head	5400630
QuantStudio 12K Flex Real-Time PCR System, 96-well block, laptop	4471050
Qubit 4.0 Fluorometer	Q33238
Bead-based extraction kits	
MagMAX Viral/Pathogen Nucleic Acid Isolation Kit	A48310
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit	Contact customer service
MagMAX DNA Multi-Sample Ultra 2.0 Kit	A36570
MagMAX Cell-Free DNA Lysis Buffer	A33600
MagMAX Cell-Free DNA Wash Buffer	A33601
MagMAX Cell-Free DNA Elution Buffer	A33602
Dynabeads MyOne SILANE Beads	37002D
MagMAX Cell-free Total Nucleic Acid Isolation Kit	A36716
MagMAX *mir*Vana Total RNA Isolation Kit	A27828
MagMAX FFPE DNA/RNA Ultra Kit	A31881
Assays and reagents	
Qubit dsDNA HS Kit	Q32854
Qubit RNA HS Assay Kit	Q32855
TaqMan Universal Master Mix II, no UNG	4440040
SuperScript VILO Master Mix	11755050
TaqMan GAPDH_m1	Hs99999905_m1
TaqMan ACTB_m1	Hs99999903_m1
TaqMan GAPDH_g1	Hs02758991_g1
TaqMan hsa-mir-16 primer set	000391
Instrument accessories and consumables	
KingFisher Flex 96 deep-well head	24079930
KingFisher Apex 96 deep-well heating block	24075930
KingFisher Flex 24 Combi head	24079940
KingFisher Apex 24 deep-well heating block	24075940
KingFisher 96 microplate (200 µL), barcoded	97002540B
KingFisher 24 deep-well plate, barcoded	95040470B
KingFisher Apex 96 Combi tip comb	97002570
KF Apex 96 Combi head	24089920
KF Apex 96 deep-well head	24079930
KF Apex 24 Combi head	24079940
KF Apex 96 KF heating block	24075920

Product	Cat. No.
Instrument accessories and consumables (continued)	
KingFisher Apex 96 deep-well heating block	24075930
KingFisher Apex 24 deep-well heating block	24075940
KingFisher Apex 96 storage tube heating block	24075950
KingFisher Apex 24 storage tube heating block	24075960
KingFisher Apex 24 storage tube adapter	N21445
KingFisher Apex 96 PCR semi skirted plate adapter	N21446
KingFisher Apex UV lamp	N21447
KingFisher 96 deep-well plates (barcoded)	9540450
KingFisher 24 deep-well plates (barcoded)	9540470
KingFisher Plastics for 96 deep-well tip combs (barcoded)	97002534
KingFisher Plastics for 24 deep-well tip combs (barcoded)	97002610

Ordering information

KingFisher Apex instruments	Cat. No.
KingFisher Apex instrument with 96 PCR head	5400910
KingFisher Apex instrument with 96 Combi head	5400920
KingFisher Apex instrument with 96 deep-well head	5400930
KingFisher Apex instrument with 24 Combi head	5400940
Plastics for 96 deep-well format	
KingFisher Apex 96 Combi tip comb	97002570
KingFisher 96 deep-well plate, barcoded	95040450B
KingFisher 96 deep-well plate, sterile, barcoded	95040460B
KingFisher 96 tip comb for deep-well magnets, barcoded	97002534B
KingFisher 96 deep-well tip comb and plate, sterile, barcoded	97002820B
Nunc Coded Cryobank Vial Systems (96 tubes)	374086
Plastics for 96 standard and PCR formats	
KingFisher Apex 96 PCR tip comb	97002560
KingFisher 96 microplate (200 µL), barcoded	97002540B
Armadillo PCR Plate, 96-well, clear, clear wells	AB2396
KingFisher 96 tip comb for KingFisher magnets, barcoded	97002524B
Armadillo PCR Plate, 96-well, clear, semi-skirted, low profile, clear wells	AB2496
Plastics for 24 deep-well format	
KingFisher Apex 24 Combi tip comb	97002580
KingFisher 24 deep-well plate, barcoded	95040470B
KingFisher 24 deep-well plate, sterile, barcoded	95040480B
KingFisher 24 deep-well tip comb and plate, barcoded	97002610B
KingFisher 24 deep-well tip comb and plate, sterile, barcoded	97002620B
Nunc 24 storage tubes	374323

KingFisher Apex accessories	Cat. No.
KingFisher Apex magnet heads	
KingFisher Apex 96 PCR head	24079910
KingFisher Apex 96 deep-well head	24079930
KingFisher Apex 96 Combi head	24079920
KingFisher Apex 24 Combi head	24079940

Ordering information

KingFisher Apex accessories (continued)	Cat. No.
KingFisher Apex heating blocks	
KingFisher Apex PCR heating block	24075910
KingFisher Apex 96 KingFisher heating block	24075920
KingFisher Apex 96 deep-well heating block	24075930
KingFisher Apex 24 deep-well heating block	24075940
KingFisher Apex 96 storage tube heating block	24075950
KingFisher Apex 24 storage tube heating block	24075960
Other	
KingFisher Apex 24 storage tube adapter	N21445
KingFisher Apex 96 PCR semi-skirted plate adapter	N21446
KingFisher Apex UV lamp	N21447

Find out more at **thermofisher.com/kingfisherapex**

ThermoFisher SCIENTIFIC