Nucleic acid isolation

# Comparison of nucleic acid extraction efficiency

## Magnetic bead–based extraction using the KingFisher Duo Prime Purification System vs. spin column–based extraction


### Summary

- Magnetic bead–based extraction using the KingFisher Duo Prime Purification System reduces hands-on time compared to spin columns
- Similar yields for DNA and higher-quality RNA were obtained using the KingFisher Duo Prime system, especially with low sample inputs
- MagMAX extraction kits have multi-sample capabilities for high-throughput processing


### Introduction

Nucleic acid extraction is routinely utilized for molecular analyses of biological samples. The accurate measurement of nucleic acid is dependent on the method used for purification. Solid-phase extraction using spin columns or magnetic beads is commonly used for nucleic acid extraction. Magnetic bead–based extraction using Thermo Scientific™ KingFisher™ instruments provides high-throughput, semi-automated solutions for the isolation of DNA and RNA from a variety of samples. Magnetic bead–based extraction kits paired with the Thermo Scientific™ KingFisher™ Duo Prime Purification System allow for a small-scale, semi-automated workflow solution for multiple sample types with minimal hands-on time. In this study, we show that the KingFisher Duo Prime system achieves nucleic acid extraction with consistent yield and quality compared to spin column extraction.

### Materials and methods

#### Sample preparation and nucleic acid quality

Whole blood samples were collected from two donors; DNA was extracted using previously frozen blood samples while RNA was extracted from fresh blood samples. Three different adherent cell lines related to lung cancer were purchased from ATCC. NCI-H1650 (ATCC, Cat. No. CRL-5883) and NCI-H1781 (ATCC, Cat. No. CRL-5894) were used for DNA extraction, and NCI-H23 (ATCC, Cat. No. CRL-5800) and NCI-H1781 were used for RNA extraction. Rabbit liver and heart were used for tissue nucleic acid extraction. Magnetic bead–based DNA extraction was performed with the Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kit and the KingFisher Duo Prime system, using DNA extraction protocols specific for each sample type. Magnetic bead–based extraction of total RNA (including small RNA) was performed with the Applied Biosystems™ MagMAX™ *mir*Vana™ Total RNA Isolation Kit. Spin column extractions for RNA and DNA were performed using kits for each sample type from supplier M and supplier Q, following the manufacturers' instructions.

DNA and RNA extractions from whole blood were performed using sample inputs of 50 µL and 100 µL. DNA and RNA extractions from cells were performed using sample inputs of 10,000 and 100,000 cells. DNA and RNA extractions from tissue were performed using 2.5 mg and 10 mg sample inputs for both rabbit liver and heart. The yield, quality, and purity of the extracted DNA were analyzed on the Thermo Scientific™ NanoDrop™ 8000 Spectrophotometer. The purity of the extracted DNA was determined by $A_{260}/A_{280}$ and $A_{260}/A_{230}$. RNA yield was analyzed on the NanoDrop 8000 Spectrophotometer. RNA quality was measured on the Agilent™ 2100 Bioanalyzer instrument. An RT-qPCR assay was used to determine the quality of RNA for downstream gene expression studies and for detection of gDNA contamination.

### RT-qPCR analysis of gene expression

Gene expression levels were evaluated using one-step RT-qPCR with fast cycling conditions on the Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System equipped with a 384-well block. Briefly, 2 µL of the purified nucleic acid from cell or blood samples; or 2 µL of the purified nucleic acid from heart tissue, diluted 10-fold; or 2 µL of the purified nucleic acid from liver tissue, diluted 100-fold, was added to a mixture containing 5 µL Applied Biosystems™ TaqMan™ Fast Virus 1-Step Master Mix and 1 µL of TaqMan™ Gene Expression Assay formulation (20X), for a 20 µL reaction. The cycling conditions were: 1 RT cycle at 50°C for 5 min, 1 cycle of RT inactivation and initial denaturation at 95°C for 20 sec, and then 40 cycles of activation at 95°C for 3 sec and amplification at 60°C for 30 sec. The qPCR reactions were run in duplicate, and the data were analyzed using automatic threshold and baseline settings.

### Evaluation of genomic DNA in RNA samples

Genomic DNA (gDNA) contamination in RNA samples was evaluated using Invitrogen™ SuperScript™ IV VILO Master Mix. The SuperScript IV VILO kit comes with a master mix that contains the RT enzyme (+RT) and a master mix without the RT enzyme (–RT), which allows for the detection of gDNA in an RNA sample. Both +RT reactions and –RT reactions were made for each RNA sample. The +RT and –RT reactions were then evaluated with a qPCR assay targeting an exon, so it can detect both RNA and gDNA. Briefly, from each RT reaction 2 µL was added to a mixture containing 5 µL TaqMan Fast Advanced Master Mix and 1 µL TaqMan Gene Expression Assay formulation (20X), for a 20 µL reaction. The cycling conditions were: 1 cycle at 50°C for 2 min, 1 cycle at 95°C for 20 sec, and then 40 cycles of activation at 95°C for 1 sec and amplification at 60°C for 20 sec.

## Results

### DNA isolation using MagMAX DNA Multi-Sample Ultra 2.0 Kit on the KingFisher Duo Prime system

Taking all three sample types into account, magnetic bead–based extraction is an efficient and easily automatable alternative to spin column extraction (Figure 1). However, differences were apparent among sample types. For instance, magnetic bead–based extraction showed yields comparable to those of supplier M kits for cells, while supplier Q kits showed higher yields for cell inputs. For rabbit heart and liver tissue, magnetic bead–based extraction resulted in superior yields compared to spin columns from both suppliers. All kits showed similar extraction efficiency for DNA from whole blood samples. The purity of the extracted DNA, determined by $A_{260}/A_{280}$ and $A_{260}/A_{230}$, was generally equivalent from all sample types for all extraction methods (Figure 2).

### RNA isolation using MagMAX *mir*Vana Total RNA Isolation Kit on the KingFisher Duo Prime system

Magnetic bead–based extraction using the MagMAX *mir*Vana kit showed higher RNA yields from tissue and whole blood samples than spin column extractions, while comparable yields were obtained from cells (Figure 3). The purity of the extracted RNA was determined using the Agilent 2100 Bioanalyzer instrument. The observed electropherograms showed significant differences in the quality of RNA from the different extraction methods. First, the MagMAX *mir*Vana kit yielded higher-quality RNA and small RNA compared to spin column extractions (Figure 4). While all methods resulted in quality RNA, spin column extraction, particularly with the spin column from supplier Q, resulted in additional peaks in the electropherograms for cells and whole blood, suggesting the presence of gDNA contamination (Figure 4).

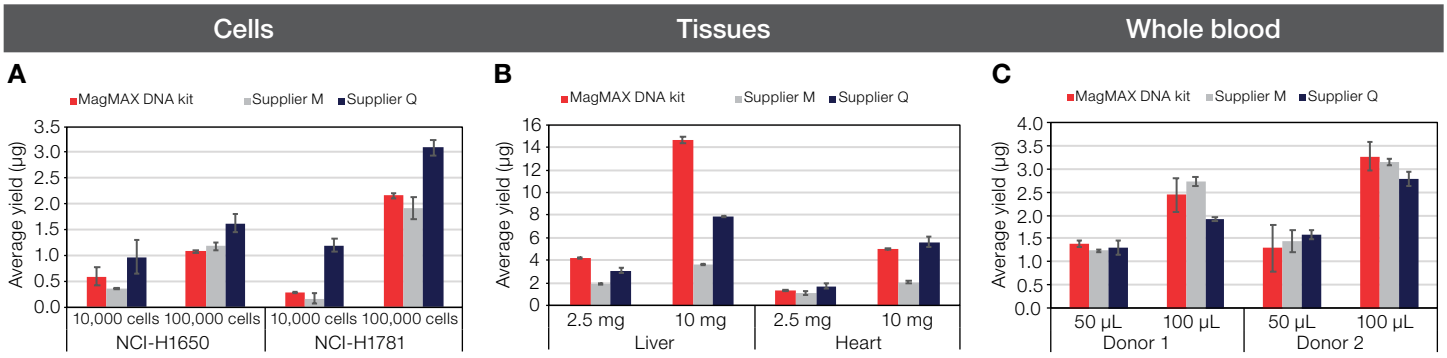


**Figure 1. DNA yields from (A) cells, (B) tissues, and (C) whole blood using the MagMAX DNA Multi-Sample Ultra 2.0 Kit on the KingFisher Duo Prime instrument.**

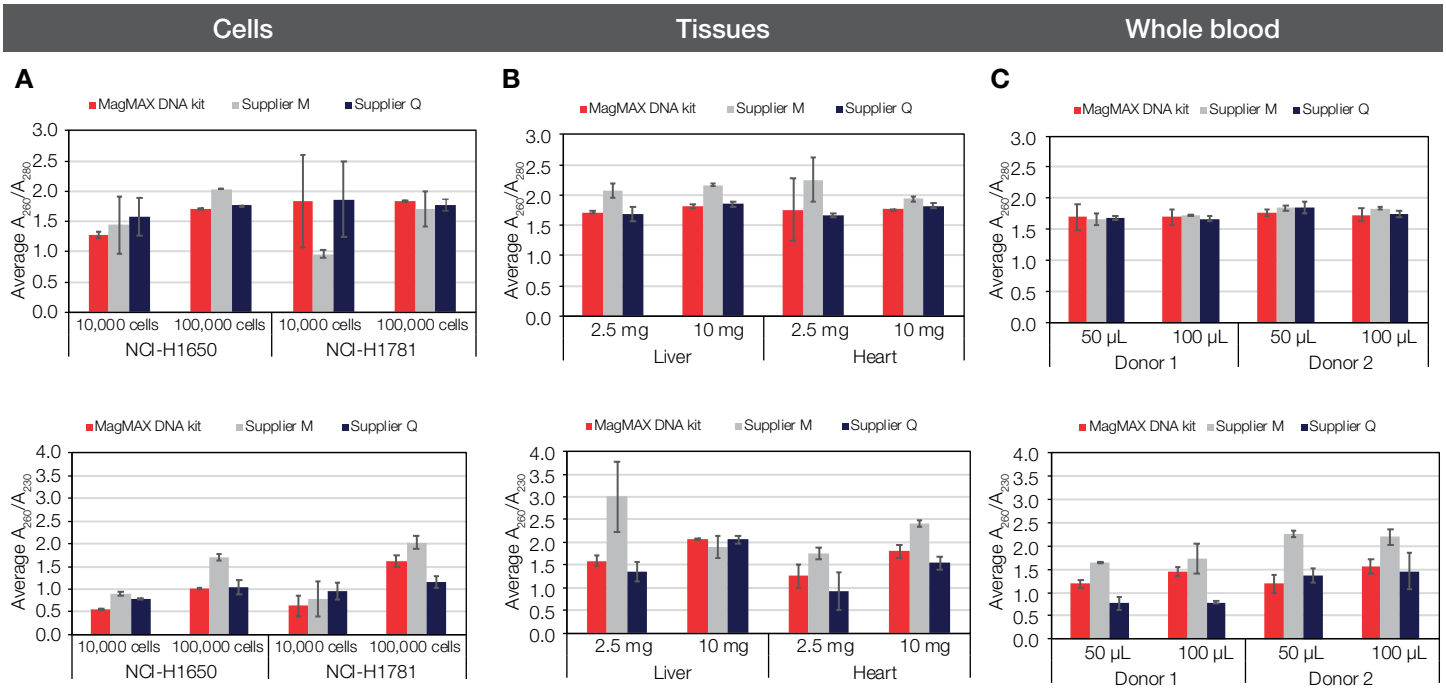


**Figure 2. Purity of extracted DNA from cells, tissues, and whole blood.** $A_{260}/A_{280}$ and $A_{260}/A_{230}$ values were calculated for DNA purified from **(A)** cells, **(B)** tissues, and **(C)** whole blood using the MagMAX DNA Multi-Sample Ultra 2.0 Kit on the KingFisher Duo Prime instrument.

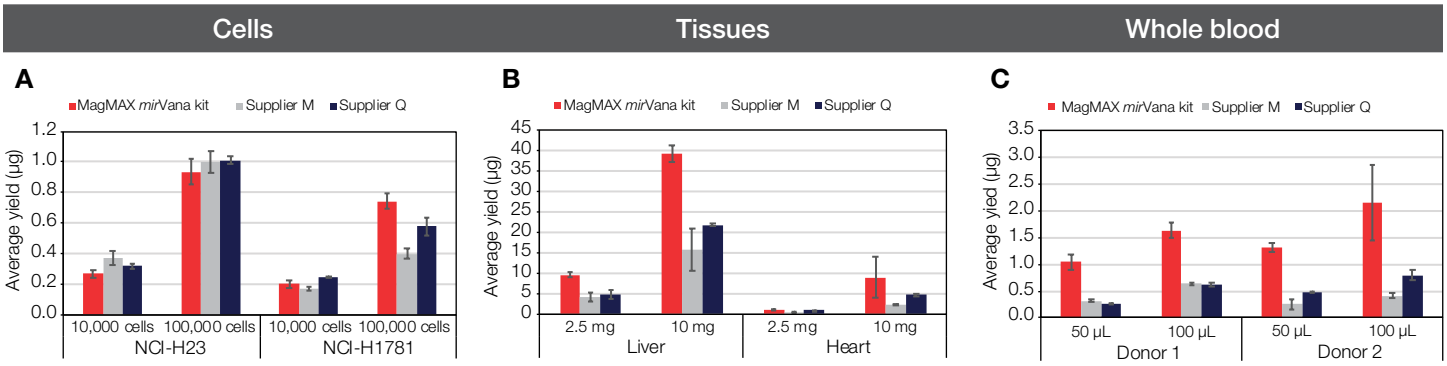


**Figure 3. RNA yields from cells, tissues, and whole blood using the MagMAX *mir*Vana Total RNA Isolation Kit on the KingFisher Duo Prime instrument.** Comparable RNA yields were obtained from **(A)** cells, while higher yields were obtained from **(B)** tissues and **(C)** blood using magnetic bead–based extraction, compared to spin column extraction.

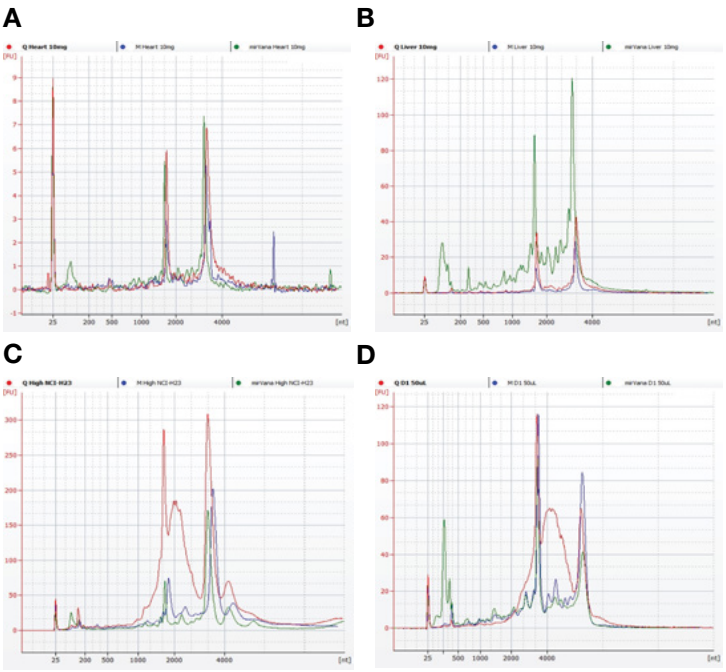


**Figure 4. RNA quality from cells, tissues, and whole blood was analyzed using the Agilent 2100 Bioanalyzer instrument.** Total RNA, including small RNA, extracted from **(A)** heart and **(B)** liver tissue using the MagMAX *mir*Vana Total RNA Isolation Kit were of higher quality than RNA extracted using spin columns. Additionally, the MagMAX *mir*Vana kit was used to isolate **(C)** cellular RNA that was free of gDNA contamination and **(D)** RNA from a low volume of whole blood.

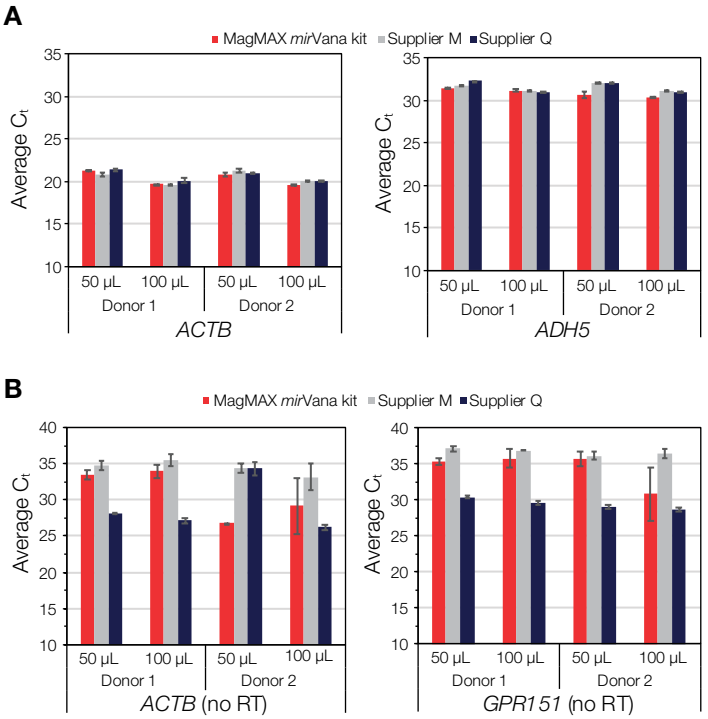


**Figure 5. Gene expression and gDNA contamination analysis of RNA purified from blood samples. (A)** Similar gene expression levels were observed with RNA isolated from blood samples using the three different kits. **(B)** gDNA contamination analysis showed the lowest $C_t$ values for RNA isolated using supplier Q's spin column, indicating high gDNA contamination.

Looking at the housekeeping genes *ACTB* and *ADH5* for the purpose of downstream gene expression analysis, using RNA from blood samples, there were no significant differences in observed $C_t$ values (Figure 5A). However, RNA isolated using the spin column from supplier Q had gDNA contamination, and thus lower $C_t$ values. RNA isolated using the MagMAX *mir*Vana kit and the spin column from supplier M was free of gDNA contamination (Figure 5B). This is consistent with the presence of additional gDNA traces in the electropherograms observed on the Agilent 2100 Bioanalyzer instrument.

Similar gene expression levels were observed with *ACTB* and *ADH5* from RNA isolated from cells. Comparable $C_t$ values were observed for all three extractions (Figure 6A). However, RNA isolated using the MagMAX *mir*Vana kit had the least gDNA contamination (Figure 6B), as seen by its highest $C_t$ values. For tissue samples, the observed $C_t$ values for *ACTB* and *ADH5* were lower for RNA extracted using the MagMAX *mir*Vana kit, indicating higher yield (Figure 7).

Spin columns and magnetic bead–based extraction efficiently extracted RNA as required for most downstream gene expression applications, suggesting that RNA yield is unlikely to be a limiting factor for the kits evaluated. However, the variation in quality of the RNA extracted using the different kits may impact downstream applications.

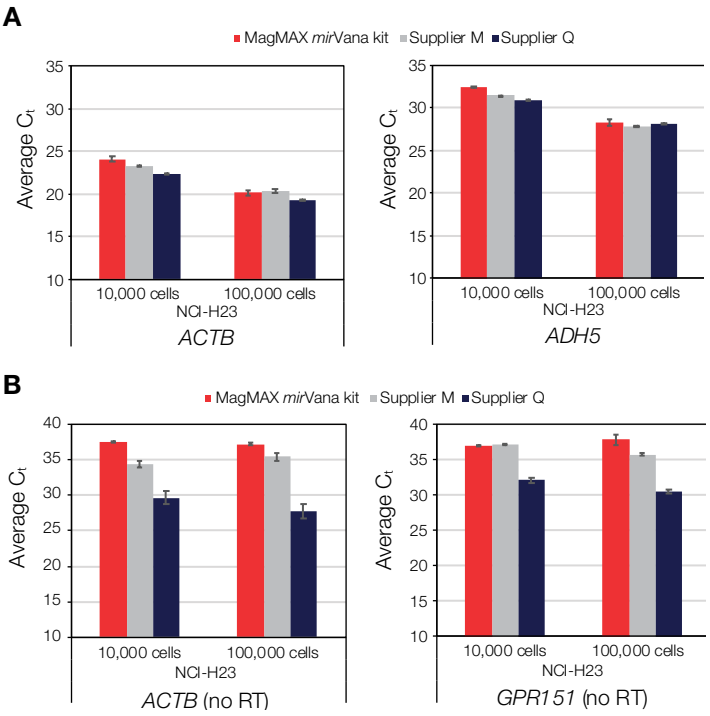


**Figure 6. Gene expression analysis and gDNA contamination analysis of RNA purified from cells. (A)** Similar gene expression levels were observed with RNA isolated from cells using the three different kits. **(B)** gDNA contamination analysis showed the lowest $C_t$ values for RNA isolated using supplier Q's spin column, indicating high gDNA contamination.

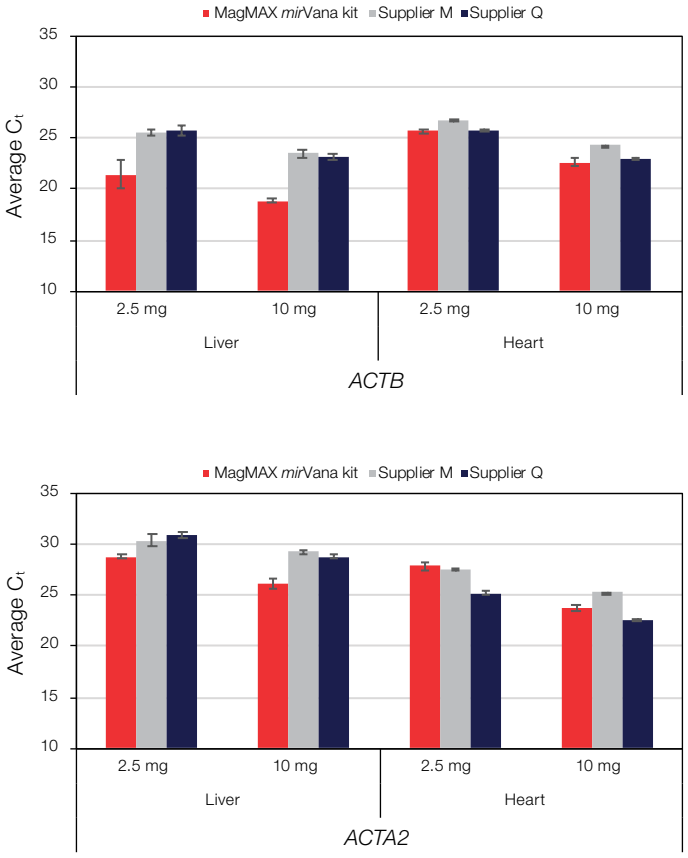


**Figure 7. Gene expression analysis of RNA purified from liver and heart tissue samples.** The RNA isolated using the MagMAX *mir*Vana kit showed lower $C_t$ values, indicating higher yields in comparison to spin columns.

## Conclusions

This study demonstrated that there are practical differences in workflows and hands-on time between commercially available spin column–based and magnetic bead–based DNA and RNA extractions. Evaluation of DNA extraction using the MagMAX DNA Multi-Sample Ultra 2.0 Kit compared to spin columns showed that sufficient yields of quality DNA can be obtained for downstream applications by magnetic bead–based extraction. For RNA extraction, both magnetic bead–based and spin column–based methods yielded amplifiable RNA, but the MagMAX *mir*Vana Total RNA Isolation Kit yielded higher-quality RNA free of gDNA contamination. Our study demonstrated that the magnetic bead–based workflow using the scalable and automation-compatible KingFisher Duo Prime Purification System enables a flexible and time-saving method to extract nucleic acid from a variety of samples.

## Ordering information

| Product | Quantity | Cat. No. |
|---|---|---|
| MagMAX DNA Multi-Sample Ultra 2.0 Kit | 1 kit | A36570 |
| MagMAX DNA Multi-Sample Ultra 2.0 Kit (with MagMAX DNA Cell and Tissue Extraction Buffer for high-throughput isolation of DNA from tissues) | 1 kit | A45721 |
| MagMAX *mir*Vana Total RNA Isolation Kit | 96 reactions | A27828 |
| KingFisher Duo Prime Purification System | 1 instrument | 5400110 |
| NanoDrop 8000 Spectrophotometer | 1 instrument | ND-8000-GL |
| TaqMan Fast Virus 1-Step Master Mix | 1 x 1 mL<br>5 x 1 mL<br>1 x 10 mL | 4444432<br>4444434<br>4444436 |
| TaqMan Gene Expression Assay | Variable | 4331182 |
| SuperScript IV VILO Master Mix | 50 reactions | 11756050 |
| TaqMan Fast Advanced Master Mix | 1 x 5 mL | 4444557 |
| QuantStudio 5 Real-Time PCR System | 1 instrument | Go to thermofisher.com/quantstudio |

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


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