

# MagMAX™ Sequential DNA/RNA Kit

For automated isolation of DNA and RNA from whole blood or bone marrow

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**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

## Product description

The Applied Biosystems™ MagMAX™ Sequential DNA/RNA Kit is developed for scalable, rapid purification of high-quality genomic DNA and total RNA from the same whole blood or bone marrow samples. DNA or RNA purified with this kit can be used in a broad range of molecular biology downstream applications, such as sequencing, genotyping, and qPCR. The kit is especially suitable for multi-omics assays, particularly when specimens are limited and/or heterogeneous. This protocol guides the user through automated sequential isolation of DNA and RNA from the same whole blood or bone marrow sample, into separate eluate solutions using KingFisher™ Flex instruments, KingFisher™ Apex systems, and KingFisher™ Duo Prime instruments.

## Workflow

Protocol duration: ~2.5 hours  
Total hands on time: ~45 minutes

Automation is compatible with the following instruments:



KingFisher™  
Duo Prime



KingFisher™  
Flex

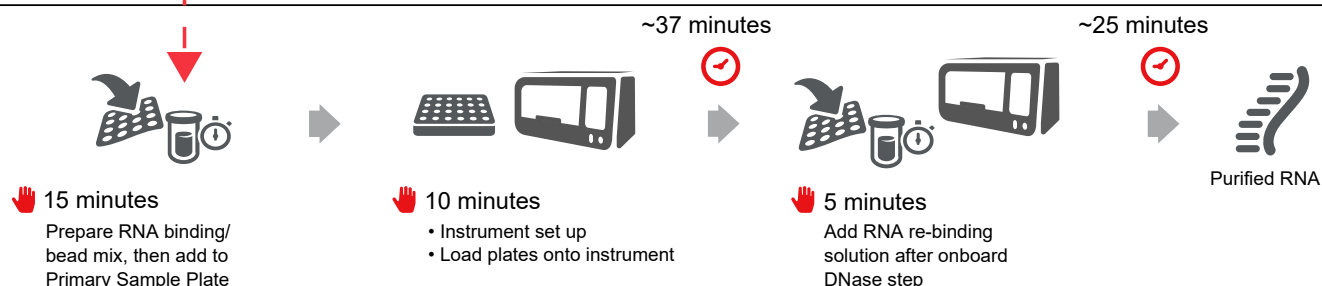


KingFisher™  
Apex Systems

DNA workflow



RNA workflow



\* Sample addition time may vary depending on the number of samples and method used to add samples to the sample plate.

## Contents and storage

Reagents provided in the kit are sufficient for 100 reactions using small (50–150 µL) sample volume inputs. If additional reagents are required for using larger (up to 500 µL) sample volume inputs for purification, see “Standalone products” on page 22 for ordering information.

**Note:** Reagent caps have color coded labels to distinguish DNA (green), RNA (blue), and universal (white) workflow components, enabling quick and easy identification, while also reducing potential errors.

Component	Quantity	Storage
Proteinase K Digestion Buffer	6 mL	15–25°C
Proteinase K (50 mg/mL)	1.5 mL	
Binding Beads <sup>[1]</sup>	4 mL	
DNA Lysis/Binding Solution	20 mL	
DNA Wash Solution I	75 mL	
DNA Elution Buffer	10 mL	
RNA Binding Solution	30 mL	
RNA Wash Solution I Concentrate <sup>[2]</sup>	115.5 mL	
RNA Elution Buffer	8 mL	
DNase Buffer	1 mL	
DNase I (50 U/µL)	0.1 mL	

<sup>[1]</sup> Do not freeze.

<sup>[2]</sup> See “Prepare RNA Wash Solution I” on page 3 for instructions on preparation of RNA Wash Solution I from the concentrate.

## Required materials not supplied

Unless otherwise indicated, all materials are available through [thermofisher.com](http://thermofisher.com). “MLS” indicates that the material is available from [fisherscientific.com](http://fisherscientific.com) or another major laboratory supplier.

Item	Source
<b>Magnetic particle processor (one of the following depending on the quantity/volume of samples being processed)</b>	
KingFisher™ Duo Prime Purification System	5400110
KingFisher™ Flex Purification System with 96 Deep-Well Head	5400630
KingFisher™ Flex Purification System with 24 Deep Well Head	5400640
KingFisher™ Apex with 96 Deep Well Head	5400930
KingFisher™ Apex with 24 Combi head	5400940
<b>Equipment</b>	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Plate Shaker (capable of shaking plates at 1050 rpm)	MLS

Item	Source
<b>Materials and consumables</b>	
KingFisher™ 96 Deep-Well Plate <sup>[1,2,3]</sup>	95040450
KingFisher™ 96 KF microplate <sup>[2]</sup>	97002540
KingFisher™ 96 Deep-Well Plate, Barcoded <sup>[3]</sup>	95040450B
KingFisher™ 96 Plate (200 µL), Barcoded <sup>[3]</sup>	97002540B
KingFisher™ 24 Deep-Well Plate, Barcoded <sup>[3]</sup>	95040470B
KingFisher™ 24 deep-well plate <sup>[1,2,3]</sup>	95040470
MicroAmp™ Clear Adhesive Film	4306311
KingFisher™ 96 tip comb for deep-well magnets <sup>[2,3]</sup>	97002534
KingFisher™ Apex 96 Combi tip comb <sup>[3]</sup>	97002570
KingFisher™ 24 deep-well tip comb and plate <sup>[2]</sup>	97002610
KingFisher™ Apex 24 Combi tip comb <sup>[3]</sup>	97002580
KingFisher™ 12-tip comb for Microtiter 96 deep-well plate <sup>[1]</sup>	97003500
KingFisher™ Duo Prime 6-tip comb and 24 deep-well plate (12 pieces of 24 deep-well plates, each including 4 tip combs) (for Duo Prime only) <sup>[1]</sup>	97003510
<b>Reagents</b>	
Ethanol, 80–100% (molecular biology grade)	MLS
Isopropanol, 100% (molecular biology grade)	MLS
PBS, pH 7.4	10010023
Nuclease-Free Water (not DEPC-Treated)	AM9932
eBioscience™ 10X RBC Lysis Buffer (multi-species) <sup>[4]</sup>	00-4300-54

<sup>[1]</sup> For use with KingFisher™ Duo Prime instruments.

<sup>[2]</sup> For use with KingFisher™ Flex instruments.

<sup>[3]</sup> For use with KingFisher™ Apex instruments.

<sup>[4]</sup> For samples containing >15,000 leukocytes/µL

## General guidelines

- Perform all steps at room temperature (20–25°C), unless otherwise noted.
- PK Digestion Buffer may exhibit precipitates and high viscosity. Warm the solution at 37°C and gently mix to dissolve the precipitates and reduce viscosity. Avoid creating bubbles.
- Wash Solution I and Binding Solutions may change color or develop inert particulates that float in the solution. **Visual particulate is not a cause for concern and does not negatively affect performance.**
- Volumes for reagent mixes are given for a single well. It is recommended to prepare a master mix for larger sample numbers. To calculate volumes for master mixes, multiply the per-well volume appropriately and add 10% overage to account for differences in pipetting.
- (Optional) To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

## Guidelines for samples

- **IMPORTANT!** Sample collection using sodium heparin anticoagulant can inhibit downstream applications.
- The kit is compatible with samples that are collected in K<sub>2</sub>EDTA.
- Use samples containing <15,000 leukocytes/ $\mu$ L for optimal nucleic acid yield and quality. For samples with >15,000 leukocytes/ $\mu$ L, removal of erythrocytes using eBioscience™ 10X RBC Lysis Buffer (multi-species) (Cat. no. 00-4300-54) before performing nucleic acid purification is recommended.

## Guidelines for Proteinase K digestion

- Do not pre-mix the PK Digestion Buffer and Proteinase K.
- Do not change the order of pipetting.

## Guidelines for Binding Bead Mix

- Vortex the Binding Beads thoroughly, combine them with the Binding Solution in a nuclease-free tube, then invert the tube until homogeneous. This mixture can be stored for up to 24 hours before dispensing into the plates.
- Ensure that the beads are fully mixed within the solution during pipetting.
- Avoid creating bubbles during mixing and pipetting.

## Procedures

Six procedures are described for sample processing. Select the appropriate protocol for your application, based on instrument type, sample type, and sample input volume.

Instrument and sample volume	Page
KingFisher™ Flex protocol for 50–150 $\mu$ L for bone marrow, or 100–150 $\mu$ L for whole blood	4
KingFisher™ Apex protocol for 50–150 $\mu$ L for bone marrow, or 100–150 $\mu$ L for whole blood	7
KingFisher™ Duo Prime protocol for 50–150 $\mu$ L for bone marrow, or 100–150 $\mu$ L for whole blood	10
KingFisher™ Flex protocol for 200–500 $\mu$ L whole blood or bone marrow	13
KingFisher™ Apex protocol for 200–500 $\mu$ L whole blood or bone marrow	16
KingFisher™ Duo Prime protocol for 200–500 $\mu$ L whole blood or bone marrow	19

## Before first use of the kit

### Prepare RNA Wash Solution I

**IMPORTANT!** Isopropanol must be added to RNA Wash Solution I Concentrate before use.

1. Prepare RNA Wash Solution I from the concentrate (1:1.5 ratio of isopropanol to RNA Wash Solution I Concentrate).
  - For the RNA Wash Solution I Concentrate (115.5 mL) included in the kit, add 77 mL of isopropanol to the bottle, then mix, and store at room temperature.
  - For standalone MagMAX™ Sequential RNA Wash Solution I Concentrate (462 mL) add 308 mL of isopropanol to the bottle, then mix, and store at room temperature.
2. Mark the bottle to indicate that isopropanol has been added to the concentrate.

## Before each use of the kit

### Resuspend Binding Beads

Vortex Binding Beads to fully resuspend the beads before each use.

# Perform DNA and RNA purification using the KingFisher™ Flex instrument (50–150 µL for bone marrow; 100–150 µL for whole blood)

- 1 Set up the instrument
  1. Ensure that the instrument is set up for processing with the proper magnetic head (96 deep-well) for your application.
  2. Ensure that the proper heat block (96 deep-well) is installed for your application.
  3. Ensure that the `SEQ_DNA_Low_Flex` and `SEQ_RNA_Low_Flex` programs have been downloaded from the product page and installed onto the instrument.

- 2 Set up the processing plates (DNA)
 

Set up the DNA Wash, DNA Elution, and Tip Comb Plates outside the instrument according to the following table.

**Table 1 Processing plates for 50–150 µL sample input**

Plate position	Plate ID	Plate type	Reagent	Volume per well
2	DNA Wash Plate 1	Deep Well	DNA Wash Solution I	750 µL
3	DNA Wash Plate 2	Deep Well	80% ethanol	750 µL
4	DNA Wash Plate 3	Deep Well	80% ethanol	750 µL
5	DNA Elution Plate	Deep Well	DNA Elution Buffer	100 µL
6	DNA Tip Comb	96 Deep-well Tip Comb in a Standard Plate		

**Note:** Load the plates onto the instrument immediately after the Primary Sample Plate has been prepared in “Prepare the Primary Sample Plate and digest with Proteinase K” on page 4.

- 3 Prepare the Primary Sample Plate and digest with Proteinase K
 

Add reagents to all wells in a 96 deep-well plate (Primary Sample Plate) that will be used for sample processing.

  1. Add 60 µL of Proteinase K Digestion Buffer to the appropriate wells of the Primary Sample Plate.
  2. Add the appropriate amount of sample to the wells containing Proteinase K Digestion Buffer.
 

**Note:** For samples <100 µL, increase the sample volume to 100 µL with PBS.
  3. Add 15 µL of Proteinase K to sample wells.
  4. Seal the Primary Sample Plate with an adhesive seal and shake for 5 minutes at 1050 rpm on a plate shaker.
  5. Select the program `SEQ_DNA_Low_Flex` on the KingFisher™ Flex instrument.
  6. Start the run and load the prepared plates into position when prompted by the instrument.
  7. Proceed to “Prepare the DNA Binding Bead Mix” during the on-board proteinase K sample digestion (~20 minutes).

- 4 Prepare the DNA Binding Bead Mix
 

Prepare the DNA Binding Bead Mix according to the following table.

**Table 2 DNA Binding Bead Mix for 50–150 µL sample input (96 samples per plate)**

Component	Volume per well	Volume per plate
DNA Lysis/Binding Solution	200 µL	21.12 mL
Binding Beads	20 µL	2.11 mL
Total volume	220 µL	23.23 mL

## 5 Purify the DNA

1. When instructed by the instrument (~20 minutes after the run has started), remove the Primary Sample Plate and add 220 µL DNA Binding Bead Mix to each sample.

**Note:** Remix the DNA Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. The mixture is viscous, pipet slowly to ensure that the correct amount is added.

**IMPORTANT!** Avoid creating bubbles when mixing and transferring reagents.

2. Immediately place the Primary Sample Plate back onto the instrument and follow the prompts on the instrument to allow the sample processing to proceed.
3. At the end of the instrument run, remove the plates from the instrument.

**IMPORTANT!** Save the Primary Sample Plate for the RNA extraction workflow.

4. Transfer the DNA eluate from the DNA Elution Plate to the final tube/plate of choice for final storage.  
The purified DNA is ready for immediate use. Alternatively, store at –20°C for long-term storage.
5. Proceed to “Prepare the DNase Solution”, followed by “Set up the processing plates (RNA)” on page 5 during the on-board DNA purification process to continue with RNA extraction.

## 6 Prepare the DNase Solution

Prepare the DNase Solution according to the following table.

**Table 3 DNase Solution for 50–150 µL sample input (96 samples per plate)**

Component	Volume per well	Volume per plate
DNase I	1 µL	0.106 mL
DNase Buffer	10 µL	1.06 mL
Nuclease-free Water	89 µL	9.434 mL
Total volume	100 µL	10.6 mL

## 7 Set up the processing plates (RNA)

Set up the RNA Wash, Ethanol Wash, DNase Treatment, RNA Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate position	Plate ID	Plate type	Reagent	Volume per well
2	RNA Wash Plate 1	Deep Well	RNA Wash Solution I <sup>[1]</sup>	1000 µL
3	RNA Wash Plate 2	Deep Well	80% ethanol	1000 µL
4	DNase Treatment Plate	Deep Well	DNase Solution	100 µL
5	RNA Wash Plate 3	Deep Well	RNA Wash Solution I <sup>[1]</sup>	750 µL
6	RNA Wash Plate 4	Deep Well	80% ethanol	750 µL
7	RNA Elution Plate	Deep Well	RNA Elution Buffer	80 µL
8	RNA Tip Comb	Place a 96 Deep-well Tip Comb in a Standard Plate		

<sup>[1]</sup> See “Prepare RNA Wash Solution I” on page 3.

**Note:** Load the plates onto the KingFisher™ Flex instrument immediately after the Primary Sample Plate has been prepared in “Purify the RNA”.

## 8 Purify the RNA

1. Prepare the RNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate
RNA Binding Solution	100 µL	10.56 mL
Isopropanol, 100%	400 µL	42.24 mL
Binding Beads	20 µL	2.11 mL
Total volume	520 µL	54.91 mL

**Note:** Remix the RNA Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. The mixture is viscous, pipet slowly to ensure that the correct amount is added.

2. Add 520 µL of RNA Binding Bead Mix to each sample in the Primary Sample Plate (saved from the DNA workflow).
3. Select the program `SEQ_RNA_Low_Flex` on the KingFisher™ Flex instrument.
4. Start the run and load the prepared plates into position when prompted by the instrument.
5. During the instrument run, prepare the RNA Re-Binding Solution according to the following table.

**Table 4 RNA Re-Binding Solution for 50–150 µL sample input (96 samples per plate)**

Component	Volume per well	Volume per plate
RNA Binding Solution	200 µL	21.12 mL
Isopropanol, 100%	250 µL	26.40 mL
Total volume	450 µL	47.52 mL

6. When instructed by the instrument (~33 minutes after the RNA run has started), take out the DNase Treatment Plate and add 450 µL of RNA Re-Binding Solution to each sample well.
7. Immediately place the plate back onto the KingFisher™ Flex instrument and follow the prompts on the instrument.
8. At the end of the run, immediately remove the plates from the instrument and transfer the RNA eluate to the final tube/plate of choice for final storage.  
The purified RNA is ready for immediate use. Alternatively, store at  $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$  for long-term storage.

# Perform DNA and RNA purification using the KingFisher™ Apex instrument (50–150 µL for bone marrow; 100–150 µL for whole blood)

- 1 Set up the instrument
  1. Ensure that the instrument is set up for processing with the proper magnetic head (96 deep-well) for your application.
  2. Ensure that the proper heat block (96 deep-well) is installed for your application.
  3. Ensure that the `SEQ_DNA_Low_Apex` and `SEQ_RNA_Low_Apex` programs have been downloaded from the product page and installed onto the instrument.

- 2 Set up the processing plates (DNA)
 

Set up the DNA Wash, DNA Elution, and Tip Comb Plates outside the instrument according to the following table.

**Table 5 Processing plates for 50–150 µL sample input**

Plate position	Plate ID	Plate type	Reagent	Volume per well
3	DNA Wash Plate 1	Deep Well	DNA Wash Solution I	750 µL
4	DNA Wash Plate 2	Deep Well	80% ethanol	750 µL
5	DNA Wash Plate 3	Deep Well	80% ethanol	750 µL
6	DNA Elution Plate	Deep Well	DNA Elution Buffer	100 µL
1	DNA Tip Comb	96 Deep-well Tip Comb in a Standard Plate		

**Note:** Load the plates onto the instrument immediately after the Primary Sample Plate has been prepared in “Prepare the Primary Sample Plate and digest with Proteinase K”.

- 3 Prepare the Primary Sample Plate and digest with Proteinase K
 

Add reagents to all wells in a 96 deep-well plate (Primary Sample Plate) that will be used for sample processing.

  1. Add 60 µL of Proteinase K Digestion Buffer to the appropriate wells of the Primary Sample Plate.
  2. Add the appropriate amount of sample to the wells containing Proteinase K Digestion Buffer.
 

**Note:** For samples <100 µL, increase the volume to 100 µL with PBS.
  3. Add 15 µL of Proteinase K to sample wells.
  4. Seal the Primary Sample Plate with an adhesive seal and shake for 5 minutes at 1050 rpm on a plate shaker.
  5. Select the program `SEQ_DNA_Low_Apex` on the KingFisher™ Apex instrument.
  6. Start the run and load the prepared plates into position when prompted by the instrument.
  7. Proceed to “Prepare the DNA Binding Bead Mix” during the on-board proteinase K sample digestion (~20 minutes).

- 4 Prepare the DNA Binding Bead Mix
 

Prepare the DNA Binding Bead Mix according to the following table.

**Table 6 DNA Binding Bead Mix for 50–150 µL sample input (96 samples per plate)**

Component	Volume per well	Volume per plate
DNA Lysis/Binding Solution	200 µL	21.12 mL
Binding Beads	20 µL	2.11 mL
Total volume	220 µL	23.23 mL

## 5 Purify the DNA

1. When instructed by the instrument (~20 minutes after the run has started), remove the Primary Sample Plate and add 220 µL DNA Binding Bead Mix to each sample.

**Note:** Remix the DNA Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. The mixture is viscous, pipet slowly to ensure that the correct amount is added.

**IMPORTANT!** Avoid creating bubbles when mixing and transferring reagents.

2. Immediately place the Primary Sample Plate back onto the instrument and follow the prompts on the instrument to allow the sample processing to proceed.
3. At the end of the instrument run, remove the plates from the instrument.

**IMPORTANT!** Save the Primary Sample Plate for the RNA extraction workflow.

4. Transfer the eluate from the DNA Elution Plate to the final tube/plate of choice for final storage. The purified DNA is ready for immediate use. Alternatively, store at –20°C for long-term storage.
5. Proceed to “Prepare the DNase Solution”, followed by “Set up the processing plates (RNA)” during the on-board DNA purification process to continue with RNA extraction.

## 6 Prepare the DNase Solution

Prepare the DNase Solution according to the following table.

**Table 7 DNase Solution for 50–150 µL sample input (96 samples per plate)**

Component	Volume per well	Volume per plate
DNase I	1 µL	0.106 mL
DNase Buffer	10 µL	1.06 mL
Nuclease-free Water	89 µL	9.434 mL
Total volume	100 µL	10.6 mL

## 7 Set up the processing plates (RNA)

Set up the RNA Wash, Ethanol Wash, DNase Treatment, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate position	Plate ID	Plate type	Reagent	Volume per well
3	RNA Wash Plate 1	Deep Well	RNA Wash Solution I <sup>[1]</sup>	1000 µL
4	RNA Wash Plate 2	Deep Well	80% ethanol	1000 µL
5	DNase Treatment Plate	Deep Well	DNase Solution	100 µL
6	RNA Wash Plate 3	Deep Well	RNA Wash Solution I <sup>[1]</sup>	750 µL
7	RNA Wash Plate 4	Deep Well	80% ethanol	750 µL
8	RNA Elution Plate	Deep Well	RNA Elution Buffer	80 µL
1	RNA Tip Comb	Place a 96 Deep-well Tip Comb in a Standard Plate		

<sup>[1]</sup> See “Prepare RNA Wash Solution I” on page 3.

**Note:** Load the plates onto the KingFisher™ Apex instrument immediately after the Primary Sample Plate has been prepared in “Purify the RNA” on page 9.



## 8 Purify the RNA

1. Prepare the RNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate
RNA Binding Solution	100 $\mu$ L	10.56 mL
Isopropanol, 100%	400 $\mu$ L	42.24 mL
Binding Beads	20 $\mu$ L	2.11 mL
Total volume	520 $\mu$ L	54.91 mL

**Note:** Remix the RNA Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. The mixture is viscous, pipet slowly to ensure that the correct amount is added.

2. Add 520  $\mu$ L of RNA Binding Bead Mix to each sample in the Primary Sample Plate (saved from the DNA workflow).
3. Select the program `SEQ_RNA_Low_Apex` on the KingFisher™ Apex instrument.
4. Start the run and load the prepared plates into position when prompted by the instrument.
5. During the instrument run, prepare the RNA Re-Binding Solution according to the following table.

**Table 8 RNA Re-Binding Solution for 50–150  $\mu$ L sample input (96 samples per plate)**

Component	Volume per well	Volume per plate
RNA Binding Solution	200 $\mu$ L	21.12 mL
Isopropanol, 100%	250 $\mu$ L	26.40 mL
Total volume	450 $\mu$ L	47.52 mL

6. When instructed by the instrument (~33 minutes after the RNA run has started), take out the DNase Treatment Plate and add 450  $\mu$ L of RNA Re-Binding Solution to each sample well.
7. Immediately place the plate back onto the KingFisher™ Apex instrument and follow the prompts on the instrument.
8. At the end of the run, immediately remove the plates from the instrument and transfer the RNA eluate to the final tube/plate of choice for storage.  
The purified RNA is ready for immediate use. Alternatively, store at  $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$  for long-term storage.

# Perform DNA and RNA purification using the KingFisher™ Duo Prime instrument (50–150 µL for bone marrow; 100–150 µL for whole blood)

- 1 Set up the instrument**
  - Ensure that the instrument is set up for processing with the proper magnetic head (12 pin) and heat block for your application.
  - Ensure that the `SEQ_DNA_Low_DUO` and `SEQ_RNA_Low_DUO` programs have been downloaded from the product page and installed onto the instrument.

- 2 Set up the processing plates (DNA)**

Prepare the plates according to the following tables. The Sample Row (Primary Sample Plate, Row A) will be prepared in “Prepare the Primary Sample Plate and digest with Proteinase K”.

**Table 9 First 96 Deep-well plate (Primary Sample Plate) layout (50–150 µL sample input)**

Plate row	Row ID	Reagent	Volume per well
A	Sample	Sample <sup>[1]</sup>	Varies
B <sup>[2]</sup>	—	—	—
C	—	Empty	
D	DNA Wash 1	DNA Wash Solution I	750 µL
E	DNA Wash 2	80% ethanol	750 µL
F	DNA Wash 3	80% ethanol	750 µL
G	—	Empty	
H	—	Empty	

<sup>[1]</sup> See “Prepare the Primary Sample Plate and digest with Proteinase K”

<sup>[2]</sup> Row reserved for 12-tip comb.

**Table 10 Second 96 Deep-well plate (DNA Elution Plate) layout (50–150 µL sample input)**

Plate row	Row ID	Reagent	Volume per well
A	DNA Elution	DNA Elution Buffer	100 µL

**Note:** Load the plate onto the instrument immediately after the Sample Row has been prepared in “Prepare the Primary Sample Plate and digest with Proteinase K”.

- 3 Prepare the Primary Sample Plate and digest with Proteinase K**

Add reagents to all wells in the Primary Sample Plate, Row A that will be used for sample processing.

  - Add 60 µL of Proteinase K Digestion Buffer to the appropriate wells of the Primary Sample Plate.
  - Add the appropriate amount of sample to the wells containing Proteinase K Digestion Buffer.

**Note:** For samples <100 µL, increase the volume in the well to 100 µL with PBS.
  - Add 15 µL of Proteinase K to sample wells.
  - Seal the Primary Sample Plate with an adhesive seal and shake for 5 minutes at 1050 rpm on a plate shaker.
  - Select the program `SEQ_DNA_Low_DUO` on the KingFisher™ Duo Prime instrument.
  - Place a 12 well tip comb in Row B.
  - Start the instrument run and load the prepared plates into position when prompted by the instrument.
  - Proceed to “Prepare the DNA Binding Bead Mix” on page 11 during the on-board proteinase K sample digestion (~20 minutes).

## 4 Prepare the DNA Binding Bead Mix

Prepare the DNA Binding Bead Mix according to the following table.

**Table 11 50–150 µL sample input (12 samples per plate)**

Component	Volume per well	Volume per plate
DNA Lysis/Binding Solution	200 µL	2.64 mL
Binding Beads	20 µL	0.264 mL
Total volume	220 µL	2.91 mL

## 5 Purify the DNA

- When instructed by the instrument (~20 minutes after the run has started), remove the Primary Sample Plate and add 220 µL DNA Binding Bead Mix to each sample.

**Note:** Remix the DNA Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. The mixture is viscous, pipet slowly to ensure that the correct amount is added.

- Immediately place the plate back onto the instrument and follow the prompts on the instrument to allow the sample processing to proceed.
- At the end of the instrument run, remove the plates from the instrument.

**IMPORTANT! Save the Primary Sample Plate** for the RNA extraction workflow.

- Transfer the DNA eluate from the DNA Elution Plate to the final tube/plate of choice for storage. The purified DNA is ready for immediate use. Alternatively, store at –20°C for long-term storage.
- Proceed to “Prepare the DNase Solution”, followed by “Set up the processing plates (RNA)” during the on-board DNA purification process to continue with RNA extraction.

## 6 Prepare the DNase Solution

Prepare the DNase Solution according to the following table.

**Table 12 DNase Solution for 50–150µL sample input (12 samples per plate)**

Component	Volume per well	Volume per plate
DNase I	1 µL	0.0132 mL
DNase Buffer	10 µL	0.132 mL
Nuclease-free Water	89 µL	1.1748 mL
Total volume	100 µL	1.32 mL

## 7 Set up the processing plates (RNA)

Set up the RNA Plate according to the following table.

**Table 13 Third 96 Deep-well plate (RNA Plate) layout (50–150 µL sample input)**

Plate row	Row ID	Reagent	Volume per well
A	DNase Treatment	DNase Solution	100 µL
B	RNA Wash 1	RNA Wash Solution I <sup>[1]</sup>	1000 µL
C	RNA Wash 2	80% ethanol	1000 µL
D	RNA Wash 3	RNA Wash Solution I <sup>[1]</sup>	750 µL
E	RNA Wash 4	80% ethanol	750 µL
F	RNA Elution	RNA Elution Buffer	80 µL
G	—	Empty	
H	—	Empty	

<sup>[1]</sup> See “Prepare RNA Wash Solution I” on page 3.

## 8 Purify the RNA

1. Prepare the RNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate
RNA Binding Solution	100 µL	1.32 mL
Isopropanol, 100%	400 µL	5.28 mL
Binding Beads	20 µL	0.264 mL
Total volume	520 µL	6.864 mL

**Note:** Remix the RNA Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. The mixture is viscous, pipet slowly to ensure that the correct amount is added.

2. Add 520 µL of RNA Binding Bead Mix to each sample (Primary Sample Plate, Row A that was saved from the DNA workflow).
3. Remove and discard the used tip comb from row H of the Primary Sample Plate.
4. Place a new 12 well tip comb in Row B.
5. Select the program `SEQ_RNA_Low_DUO` on the KingFisher™ Duo Prime instrument.
6. Start the instrument run and load the prepared plates into position when prompted by the instrument.
7. During the instrument run, prepare the RNA Re-Binding Solution according to the following table.

Component	Volume per well	Volume per plate
RNA Binding Solution	200 µL	2.64 mL
Isopropanol, 100%	250 µL	3.3 mL
Total volume	450 µL	5.96 mL

8. When instructed by the instrument (~33 minutes after the RNA run has started), take out the third 96 deep-well plate (RNA Plate) and add 450 µL of RNA Re-Binding Solution to each sample in Row A.
9. Immediately place the plate back onto the KingFisher™ Duo Prime instrument and follow the prompts on the instrument.
10. At the end of the run, immediately remove the plates from the instrument and transfer the RNA eluate to the final tube/plate of choice for storage.  
The purified RNA is ready for immediate use. Alternatively, store the samples at  $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$  for long-term storage.

# Perform DNA and RNA purification using the KingFisher™ Flex instrument (200–500 µL whole blood or bone marrow)

- 1 Set up the instrument**
  1. Ensure that the instrument is set up for processing with the proper magnetic head (24 deep-well) for your application.
  2. Ensure that the proper heat block (24 deep-well) is installed for your application.
  3. Ensure that the `SEQ_DNA_High_Flex` and `SEQ_RNA_High_Flex` programs have been downloaded from the product page and installed onto the instrument.

- 2 Set up the processing plates (DNA)**

Set up the DNA Wash, DNA Elution, and Tip Comb Plates outside the instrument according to the following table.

**Table 14 Processing plates 200–500 µL sample input**

Plate position	Plate ID	Plate type	Reagent	Volume per well
2	DNA Wash Plate 1	Deep Well	DNA Wash Solution I	3000 µL
3	DNA Wash Plate 2	Deep Well	80% ethanol	3000 µL
4	DNA Wash Plate 3	Deep Well	80% ethanol	3000 µL
5	DNA Elution Plate	Deep Well	DNA Elution Buffer	200–500 µL
6	DNA Tip Comb	24 Deep-well Tip Comb Plate		

**Note:** Load the plates onto the instrument immediately after the Primary Sample Plate has been prepared in “Prepare the Primary Sample Plate and digest with Proteinase K”.

- 3 Prepare the Primary Sample Plate and digest with Proteinase K**

This protocol describes procedures using four different sample volumes (200 µL, 300 µL, 400 µL, and 500 µL). For sample amounts that are less than a listed sample volume, increase the volume to a listed value with PBS, then follow the appropriate procedure. For example, a 250 µL sample would be brought up to 300 µL, and processed following the 300 µL sample volume procedure.

1. Add reagents for the appropriate sample volume to a 24 deep-well plate (Primary Sample Plate) in the following order.
  1. Proteinase K Digestion Buffer
  2. Sample
  3. Proteinase K

Use the appropriate amount of reagent for the sample volume to be added in each well according to the following table.

Proteinase K Digestion Buffer	Sample volume	Proteinase K
100 µL	200 µL	20 µL
150 µL	300 µL	30 µL
200 µL	400 µL	30 µL
250 µL	500 µL	30 µL whole blood, or 40 µL bone marrow

2. Seal the Primary Sample Plate with an adhesive seal and shake for 5 minutes at 1050 rpm on a plate shaker.
3. Select the program `SEQ_DNA_High_Flex` on the KingFisher™ Flex instrument.
4. Start the run and load the prepared plates into position when prompted by the instrument.
5. Proceed to “Prepare the DNA Binding Bead Mix” on page 14 during the on-board proteinase K sample digestion (~20 minutes).

## 4 Prepare the DNA Binding Bead Mix

Prepare the DNA Binding Bead Mix according to the following table.

**Table 15 DNA Binding Bead Mix for 200–500 µL sample input (24 samples per plate)**

Sample volume	DNA Lysis/Binding Solution	Binding Beads	DNA Binding Bead Mix
200 µL	400 µL	30 µL	430 µL
300 µL	500 µL	30 µL	530 µL
400 µL	700 µL	30 µL	730 µL
500 µL	800 µL	30 µL	830 µL

## 5 Purify the DNA

1. When instructed by the instrument (~20 minutes after the run has started), remove the Primary Sample Plate and add the appropriate amount of DNA Binding Bead Mix to each sample (See “Prepare the DNA Binding Bead Mix” on page 14).

**Note:** Remix the DNA Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. The mixture is viscous, pipet slowly to ensure that the correct amount is added.

2. Immediately place the plate back onto the instrument and follow the prompts on the instrument to allow the sample processing to proceed.
3. At the end of the instrument run, remove the plates from the instrument.

**IMPORTANT! Save the Primary Sample Plate** for the RNA extraction workflow.

4. Transfer the DNA eluate from the DNA Elution Plate to the final tube/plate of choice for storage. The purified DNA is ready for immediate use. Alternatively, store at –20°C for long-term storage.
5. Proceed to “Prepare the DNase Solution”, followed by “Set up the processing plates (RNA)” during the on-board DNA purification process to continue with RNA extraction.

## 6 Prepare the DNase Solution

Prepare the DNase Solution according to the following table.

**Table 16 DNase Solution for 200–500 µL sample input**

Component	Volume per well
DNase I	2 µL
DNase Buffer	20 µL
Nuclease-free Water	178 µL
Total volume	200 µL

## 7 Set up the processing plates (RNA)

Set up the RNA Wash, Ethanol Wash, DNase Treatment, RNA Elution, and RNA Tip Comb Plates outside the instrument according to the following table.

Plate position	Plate ID	Plate type	Reagent	Volume per well
2	RNA Wash Plate 1	Deep Well	RNA Wash Solution I <sup>[1]</sup>	4000 µL
3	RNA Wash Plate 2	Deep Well	80% ethanol	4000 µL
4	DNase Treatment Plate	Deep Well	DNase Solution	200 µL
5	RNA Wash Plate 3	Deep Well	RNA Wash Solution I <sup>[1]</sup>	2000 µL
6	RNA Wash Plate 4	Deep Well	80% ethanol	2000 µL
7	RNA Elution Plate	Deep Well	RNA Elution Buffer	100–150 µL
8	RNA Tip Comb	24 Deep-well Tip Comb Plate		

<sup>[1]</sup> See “Prepare RNA Wash Solution I” on page 3.

**Note:** Load the plates onto the instrument immediately after the Primary Sample Plate has been prepared.

## 8 Purify the RNA

1. Prepare the RNA Binding Bead Mix according to the following table.

**Table 17 RNA Binding Bead Mix for 200–500 µL sample input (24 samples per plate)**

Sample volume	RNA Binding Solution	Isopropanol, 100%	Binding Beads	RNA Binding Bead Mix
200 µL	200 µL	800 µL	30 µL	1030 µL
300 µL	300 µL	1200 µL	30 µL	1530 µL
400 µL	400 µL	1600 µL	30 µL	2030 µL
500 µL	400 µL	1600 µL	30 µL	2030 µL

**Note:** Remix the RNA Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. The mixture is viscous, pipet slowly to ensure that the correct amount is added.

2. Add the appropriate volume of RNA Binding Bead Mix to each sample in the Primary Sample Plate (saved from the DNA workflow).
3. Select the program `SEQ_RNA_High_Flex` on the KingFisher™ Flex instrument.
4. Start the instrument run and load the prepared plates into position when prompted by the instrument.
5. During the instrument run, prepare the RNA Re-Binding Solution according to the following table.

Component	Volume per well
RNA Binding Solution	400 µL
Isopropanol, 100%	500 µL
Total volume	900 µL

6. When instructed by the instrument (~33 minutes after the RNA run has started), **take out the DNase Treatment Plate** and add 900 µL of RNA Re-Binding Solution to each sample.
7. Immediately place the plate back onto the KingFisher™ Flex instrument and follow the prompts on the instrument.
8. At the end of the run, immediately remove the plates from the instrument and transfer the RNA eluate to the final tube/plate of choice for storage.  
The purified RNA is ready for immediate use. Alternatively, store the samples at  $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$ .

# Perform DNA and RNA purification using the KingFisher™ Apex instrument (200–500 µL whole blood or bone marrow)

- 1 Set up the instrument
  1. Ensure that the instrument is set up for processing with the proper magnetic head (24 deep-well) for your application.
  2. Ensure that the proper heat block (24 deep-well) is installed for your application.
  3. Ensure that the `SEQ_DNA_High_Apex` and `SEQ_RNA_High_Apex` programs have been downloaded from the product page and installed onto the instrument.

- 2 Set up the processing plates (DNA)
 

Set up the DNA Wash, DNA Elution, and Tip Comb Plates outside the instrument according to the following table.

**Table 18 Processing plates 200–500 µL sample input**

Plate position	Plate ID	Plate type	Reagent	Volume per well
3	DNA Wash Plate 1	Deep Well	DNA Wash Solution I	3000 µL
4	DNA Wash Plate 2	Deep Well	80% ethanol	3000 µL
5	DNA Wash Plate 3	Deep Well	80% ethanol	3000 µL
6	DNA Elution Plate	Deep Well	DNA Elution Buffer	200–500 µL
1	DNA Tip Comb	24 Deep-well Tip Comb Plate		

**Note:** Load the plates onto the instrument immediately after the Primary Sample Plate has been prepared in “Prepare the Primary Sample Plate and digest with Proteinase K”.

- 3 Prepare the Primary Sample Plate and digest with Proteinase K
 

This protocol describes procedures using four different sample volumes (200 µL, 300 µL, 400 µL, and 500 µL). For sample amounts that are less than a listed sample volume, increase the volume to a listed value with PBS, then follow the appropriate procedure. For example, a 250 µL sample would be brought up to 300 µL, and processed following the 300 µL sample volume procedure.

1. Add reagents for the appropriate sample volume to a 24 deep-well plate (Primary Sample Plate) in the following order.
  1. Proteinase K Digestion Buffer
  2. Sample
  3. Proteinase K

Use the appropriate amount of reagent for the sample volume to be added in each well according to the following table.

Proteinase K Digestion Buffer	Sample volume	Proteinase K
100 µL	200 µL	20 µL
150 µL	300 µL	30 µL
200 µL	400 µL	30 µL
250 µL	500 µL	30 µL whole blood, or 40 µL bone marrow

2. Seal the Primary Sample Plate with an adhesive seal and shake for 5 minutes at 1050 rpm on a plate shaker.
3. Select the program `SEQ_DNA_High_Apex` on the KingFisher™ Apex instrument.
4. Start the run and load the prepared plates into position when prompted by the instrument.
5. Proceed to “Prepare the DNA Binding Bead Mix” on page 17 during the on-board proteinase K sample digestion (~20 minutes).



#### 4 Prepare the DNA Binding Bead Mix

Prepare the DNA Binding Bead Mix according to the following table.

**Table 19 DNA Binding Bead Mix for 200–500 µL sample input (24 samples per plate)**

Sample volume	DNA Lysis/Binding Solution	Binding Beads	DNA Binding Bead Mix
200 µL	400 µL	30 µL	430 µL
300 µL	500 µL	30 µL	530 µL
400 µL	700 µL	30 µL	730 µL
500 µL	800 µL	30 µL	830 µL

#### 5 Purify the DNA

1. When instructed by the instrument (~20 minutes after the run has started), remove the Primary Sample Plate and add the appropriate amount of DNA Binding Bead Mix to each sample (See “Prepare the DNA Binding Bead Mix” on page 17).

**Note:** Remix the DNA Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. The mixture is viscous, pipet slowly to ensure that the correct amount is added.

2. Immediately place the plate back onto the instrument and follow the prompts on the instrument to allow the sample processing to proceed.
3. At the end of the instrument run, remove the plates from the instrument.

**IMPORTANT! Save the Primary Sample Plate** for the RNA extraction workflow.

4. Transfer the DNA eluate from the DNA Elution Plate to the final tube/plate of choice for storage. The purified DNA is ready for immediate use. Alternatively, store at –20°C for long-term storage.
5. Proceed to “Prepare the DNase Solution”, followed by “Set up the processing plates (RNA)” during the on-board DNA purification process to continue with RNA extraction.

#### 6 Prepare the DNase Solution

Prepare the DNase Solution according to the following table.

**Table 20 DNase Solution for 200–500 µL sample input**

Component	Volume per well
DNase I	2 µL
DNase Buffer	20 µL
Nuclease-free Water	178 µL
Total volume	200 µL

#### 7 Set up the processing plates (RNA)

Set up the RNA Wash, Ethanol Wash, DNase Treatment, RNA Elution, and RNA Tip Comb Plates outside the instrument according to the following table.

Plate position	Plate ID	Plate type	Reagent	Volume per well
3	RNA Wash Plate 1	Deep Well	RNA Wash Solution I <sup>[1]</sup>	4000 µL
4	RNA Wash Plate 2	Deep Well	80% ethanol	4000 µL
5	DNase Treatment Plate	Deep Well	DNase Solution	200 µL
6	RNA Wash Plate 3	Deep Well	RNA Wash Solution I <sup>[1]</sup>	2000 µL
7	RNA Wash Plate 4	Deep Well	80% ethanol	2000 µL
8	RNA Elution Plate	Deep Well	RNA Elution Buffer	100–150 µL
1	RNA Tip Comb	24 Deep-well Tip Comb Plate		

<sup>[1]</sup> See “Prepare RNA Wash Solution I” on page 3.

**Note:** Load the plates onto the instrument immediately after the Primary Sample Plate has been prepared.

## 8 Purify the RNA

1. Prepare the RNA Binding Bead Mix according to the following table.

**Table 21 RNA Binding Bead Mix for 200–500 µL sample input (24 samples per plate)**

Sample volume	RNA Binding Solution	Isopropanol, 100%	Binding Beads	RNA Binding Bead Mix
200 µL	200 µL	800 µL	30 µL	1030 µL
300 µL	300 µL	1200 µL	30 µL	1530 µL
400 µL	400 µL	1600 µL	30 µL	2030 µL
500 µL	400 µL	1600 µL	30 µL	2030 µL

**Note:** Remix the RNA Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. The mixture is viscous, pipet slowly to ensure that the correct amount is added.

2. Add the appropriate volume of RNA Binding Bead Mix to each sample in the Primary Sample Plate (saved from the DNA workflow).
3. Select the program `SEQ_RNA_High_Apex` on the KingFisher™ Apex instrument.
4. Start the run and load the prepared plates into position when prompted by the instrument.
5. During the run, prepare the RNA Re-Binding Solution according to the following table.

Component	Volume per well
RNA Binding Solution	400 µL
Isopropanol , 100%	500 µL
Total volume	900 µL

6. When instructed by the instrument (~33 minutes after the RNA run has started), take out the DNase Treatment Plate and add 900 µL of RNA Re-Binding Solution to each sample.
7. Immediately place the plate back onto the KingFisher™ Apex instrument and follow the prompts on the instrument.
8. At the end of the run, immediately remove the plates from the instrument and transfer the RNA eluate to the final tube/plate of choice for storage.

The purified RNA is ready for immediate use. Alternatively, store at  $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$ .

# Perform DNA and RNA purification using the KingFisher™ Duo Prime instrument (200–500 µL whole blood or bone marrow)

- 1 Set up the instrument
  1. Ensure that the instrument is set up for processing with the proper magnetic head (6 pin) and heat block for your application.
  2. Ensure that the SEQ\_DNA\_High\_DUO and SEQ\_RNA\_High\_DUO programs have been downloaded from the product page and installed onto the instrument.

- 2 Set up the processing plates (DNA)
 

Prepare the plates according to the following tables. The Sample Row (Primary Sample Plate, Row A) will be prepared in “Prepare the Primary Sample Plate and digest with Proteinase K”.

**Table 22 First 24 Deep-well plate (Primary Sample Plate) layout (200–500 µL sample input)**

Plate row	Row ID	Reagent	Volume per well
A	Sample	Sample <sup>[1]</sup>	Varies
B <sup>[2]</sup>	—	—	—
C	—	Empty	
D	—	Empty	

<sup>[1]</sup> See “Prepare the Primary Sample Plate and digest with Proteinase K”

<sup>[2]</sup> Row reserved for 6-tip comb.

**Table 23 Second 24 Deep-well plate (DNA Elution Plate) layout (200–500 µL sample input)**

Plate row	Row ID	Reagent	Volume per well
A	DNA Elution	DNA Elution Buffer	200–500 µL
B	DNA Wash 1	DNA Wash Solution I	3000 µL
C	DNA Wash 2	80% ethanol	3000 µL
D	DNA Wash 3	80% ethanol	3000 µL

**Note:** Load the plate onto the instrument immediately after the Sample Row has been prepared in “Prepare the Primary Sample Plate and digest with Proteinase K”.

- 3 Prepare the Primary Sample Plate and digest with Proteinase K
 

This protocol describes procedures using four different sample volumes (200 µL, 300 µL, 400 µL, and 500 µL). For sample amounts that are less than a listed sample volume, increase the volume to a listed value with PBS, then follow the appropriate procedure. For example, a 250 µL sample would be brought up to 300 µL, and processed following the 300 µL sample volume procedure.

1. Add reagents to the Primary Sample Plate, Row A in the following order.
  1. Proteinase K Digestion Buffer
  2. Sample
  3. Proteinase K

Use the appropriate amount of reagent for the sample volume to be added in each well according to the following table.

Proteinase K Digestion Buffer	Sample volume	Proteinase K
100 µL	200 µL	20 µL
150 µL	300 µL	30 µL
200 µL	400 µL	30 µL
250 µL	500 µL	30 µL whole blood, or 40 µL bone marrow

2. Seal the Primary Sample Plate with an adhesive seal and shake for 5 minutes at 1050 rpm on a plate shaker.

### 3 (continued)

3. Select the program `SEQ_DNA_High_DUO` on the KingFisher™ Duo Prime instrument.
4. Start the run and load the prepared plates into position when prompted by the instrument.
5. Proceed to “Prepare the DNA Binding Bead Mix” on page 20 during the on-board proteinase K sample digestion (~20 minutes).

### 4 Prepare the DNA Binding Bead Mix

Prepare the DNA Binding Bead Mix according to the following table.

**Table 24 DNA Binding Bead Mix for 200–500 µL sample input (24 samples per plate)**

Sample volume	DNA Lysis/Binding Solution	Binding Beads	DNA Binding Bead Mix
200 µL	400 µL	30 µL	430 µL
300 µL	500 µL	30 µL	530 µL
400 µL	700 µL	30 µL	730 µL
500 µL	800 µL	30 µL	830 µL

### 5 Purify the DNA

1. When instructed by the instrument (~20 minutes after the run has started), remove the Primary Sample Plate and add the appropriate amount of DNA Binding Bead Mix to each sample (See “Prepare the DNA Binding Bead Mix” on page 20).  
**Note:** Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. The mixture is viscous, pipet slowly to ensure that the correct amount is added.
2. Immediately place the plate back onto the instrument and follow the prompts on the instrument to allow the sample processing to proceed.
3. At the end of the instrument run, remove the plates from the instrument.

**IMPORTANT! Save the Primary Sample Plate** for the RNA extraction workflow.

4. Immediately transfer the DNA eluate to the final tube/plate of choice for storage.  
The purified DNA is ready for immediate use. Alternatively, store at –20°C for long-term storage.
5. Proceed to “Prepare the DNase Solution”, followed by “Set up the processing plates (RNA)” during the on-board DNA purification process to continue with RNA extraction.

### 6 Prepare the DNase Solution

During on-board DNA purification, prepare the DNase Solution according to the following table.

**Table 25 DNase Solution for 200–500 µL sample input**

Component	Volume per well
DNase I	2 µL
DNase Buffer	20 µL
Nuclease-free Water	178 µL
Total volume	200 µL

## 7 Set up the processing plates (RNA)

Set up a 24 Deep Well Plate (RNA Plate) according to the following table.

**Table 26 Third 24 Deep-well plate (RNA Plate) layout**

Plate row	Row ID	Reagent	Volume per well
A	DNase Treatment	DNase Solution	200 µL
B	RNA Wash 3	RNA Wash Solution I <sup>[1]</sup>	2000 µL
C	RNA Wash 4	80% Ethanol	2000 µL
D	RNA Elution	RNA Elution Buffer	100–150 µL

<sup>[1]</sup> See "Prepare RNA Wash Solution I" on page 3.

## 8 Purify the RNA

1. Prepare the RNA Binding Bead Mix according to the following table.

**Table 27 RNA Binding Bead Mix for 200–500 µL sample input (24 samples per plate)**

Sample volume	RNA Binding Solution	Isopropanol, 100%	Binding Beads	RNA Binding Bead Mix
200 µL	200 µL	800 µL	30 µL	1030 µL
300 µL	300 µL	1200 µL	30 µL	1530 µL
400 µL	400 µL	1600 µL	30 µL	2030 µL
500 µL	400 µL	1600 µL	30 µL	2030 µL

**Note:** Remix the RNA Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. The mixture is viscous, pipet slowly to ensure that the correct amount is added.

2. Remove the used tip comb from row D.
3. Add the appropriate volume of RNA Binding Bead Mix to each sample (Primary Sample Plate, Row A from the DNA workflow), then add reagents to the other rows according to the following table.

Plate row	Row ID	Reagent	Volume per well
A	Sample	DNA Extraction Leftover + RNA Binding Bead Mix	Varies
B	6 Well Tip Comb	—	—
C	RNA Wash 1	RNA Wash Solution I <sup>[1]</sup>	4000 µL
D	RNA Wash 2	80% Ethanol	4000 µL

<sup>[1]</sup> See "Prepare RNA Wash Solution I" on page 3.

4. Add a 6-well Tip Comb to Row B of the Primary Sample Plate.
5. Select the program `SEQ_RNA_High_DUO` on the KingFisher™ Duo Prime instrument.
6. Start the instrument run and load the prepared plates into position when prompted by the instrument.
7. During the instrument run, prepare the RNA Re-Binding Solution according to the following table.

Component	Volume per well
RNA Binding Solution	400 µL
Isopropanol, 100%	500 µL
Total volume	900 µL

8. When instructed by the instrument (~33 minutes after the RNA run has started), take out the third plate (RNA Elution Plate) and add 900 µL of RNA Re-Binding Solution to each sample in Row A.
9. Immediately place the plate back onto the KingFisher™ Duo Prime instrument and follow the prompts on the instrument.
10. At the end of the run, immediately remove the plates from the instrument and transfer the RNA eluate to the final tube/plate of choice for storage.  
The purified RNA is ready for immediate use. Alternatively, store at  $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$  for long-term storage.

## Quantitation

Use the Qubit™ fluorometer with the Qubit™ 1X dsDNA BR Assay Kit (Cat. No. [Q33266](#)) and Qubit™ RNA HS Assay Kit (Cat. No. [Q32855](#)) to determine the amount of DNA and RNA isolated from whole blood or bone marrow. Quantitation by qPCR using the Applied Biosystems™ TaqMan™ RNase P Detection Reagents Kit (Cat. No. [4316831](#)) is also an acceptable method.

## Standalone products

Unless otherwise indicated, all materials are available through [thermofisher.com](http://thermofisher.com).

Catalog numbers that appear as links open the web pages for those products.

Component	Quantity	Cat. No.
MagMAX™ Sequential DNA Lysis/Binding Solution	80 mL	<a href="#">A66591</a>
MagMAX™ Sequential RNA Binding Solution	120 mL	<a href="#">A66592</a>
MagMAX™ Sequential RNA Wash Solution I Concentrate <sup>[1]</sup>	462 mL	<a href="#">A66593</a>
MagMAX™ Sequential DNA Wash Solution I	300 mL	<a href="#">A66594</a>
MagMAX™ Sequential DNA Elution Buffer	40 mL	<a href="#">A66595</a>
MagMAX™ Sequential RNA Elution Buffer	32 mL	<a href="#">A66596</a>
MagMAX™ Sequential Proteinase K (50 mg/mL)	6 mL	<a href="#">A66597</a>
MagMAX™ Sequential Binding Beads	16 mL	<a href="#">A66598</a>
MagMAX™ Sequential Proteinase K Digestion Buffer	24 mL	<a href="#">A66599</a>
MagMAX™ Sequential DNase I (50 U/µL)	0.4 mL	<a href="#">A66600</a>
MagMAX™ Sequential DNase Buffer	4 mL	<a href="#">A66601</a>

<sup>[1]</sup> Requires addition of 100% isopropanol, sold separately.

## Limited product warranty

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Revision history: Pub. No. MAN0030308 A

Revision	Date	Description
A	13 June 2024	New document for the MagMAX™ Sequential DNA/RNA Kit.

The information in this guide is subject to change without notice.

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