MagMAX[™] FFPE DNA/RNA Ultra Kit

Automated or manual sequential isolation of DNA and RNA from FFPE samples using AutoLys tubes

Catalog Number A31881

Pub. No. MAN0017541 Rev. A.0

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**.

Product description

The Applied Biosystems[™] MagMAX[™] FFPE DNA/RNA Ultra Kit is designed to isolate both DNA and RNA from the same section of formaldehyde- or paraformaldehyde-fixed, paraffin-embedded (FFPE) tissues. The kit also allows for flexibility to isolate DNA only, RNA only or total nucleic acid (TNA). The kit uses MagMAX[™] magneticbead technology, ensuring reproducible recovery of high-quality nucleic acid through manual or automated processing. The isolated nucleic acid is appropriate for use with a broad range of downstream assays, such as quantitative real-time RT-PCR and next-generation sequencing.

In addition to the use of traditional solvents, the kit is compatible with Autolys M tubes that enable a faster and more convenient means of deparaffinizing FFPE samples by eliminating the need for organic solvents such as xylene or CitriSolv and ethanol. Samples are put into the tubes for protease digestion, tubes are lifted with the Auto-pliers or Auto-Lifter and then samples are spun down. The wax and debris are contained in the upper chamber while the lysate is passed through. Afterwards, the clarified lysate can be directly purified with the MagMAX[™] FFPE DNA/RNA Ultra Kit.

For guides without using AutoLys M tubes for sequential DNA and RNA isolation, or DNA isolation, or RNA isolation only, see *MagMAX[™] FFPE DNA/RNA Ultra Kit User Guide (sequential DNA/RNA isolation)* (Pub. No. MAN0015877), or *MagMAX[™] FFPE DNA/RNA Ultra Kit User Guide (DNA isolation only)* (Pub. No. MAN0015905), or *MagMAX[™] FFPE DNA/RNA Ultra Kit User Guide (RNA isolation only)* (Pub. No. MAN0015906), respectively.

This guide describes isolation of DNA and RNA from FFPE tissue blocks or FFPE slides using AutoLys M tubes. Three optimized methods for sections or curls both up to 40 μm using AutoLys M tubes are included:

- Manual sample processing.
- KingFisher[™] Flex Magnetic Particle Processor with 96 Deep-Well Head (DW96; 96-well deep well setting).
- KingFisher[™] Duo Prime Magnetic Particle Processor (12-well deep well setting).

For DNA isolation, or RNA isolation, or TNA isolation only, see MagMAX[™] FFPE DNA/RNA Ultra Kit User Guide (DNA isolation only) (Pub. No. MAN0017539), or MagMAX[™] FFPE DNA/RNA Ultra Kit User Guide (RNA isolation only) (Pub. No. MAN0017540), or MagMAX[™] FFPE DNA/RNA Ultra Kit User Guide (TNA isolation only) (Pub. No. MAN0017538), respectively.

Contents and storage

Reagents provided in the kit are sufficient for 48 DNA and RNA isolations from sections up to 40 μm with the AutoLys workflow.

Table 1 MagMAX[™] FFPE DNA/RNA Ultra Kit (Cat. No. A31881)

Contents	Amount	Storage	
Protease	960 µL	-25°C to -15°C	
Protease Digestion Buffer ^[1]	10 mL	15°C to 30°C	
Binding Solution ^[1]	38.5 mL		
Nucleic Acid Binding Beads ^[2]	1.95 mL	2°C to 8°C	
DNA Wash Buffer	38.5 mL		
Wash Solution 2 Concentrate	210 mL ^[3]	- 15°C to 30°C	
Elution Solution	5 mL		
RNA Wash Buffer Concentrate	115 mL ^[3]		
DNase	1.95 mL	25°C to -15°C	
DNase buffer	960 µL	-25 0 10 -15 0	

^[1] Additional reagents are available separately; Protease Digestion Buffer, Binding Solution, and DNA Wash Buffer are also available as Cat. No. A32796.

^[2] Shipped at room temperature.

^[3] Final volume; see "Isolate DNA and RNA" on page 4.

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. MLS: Fisher Scientific (fisherscientific.com) or other major laboratory supplier.

 Table 2
 Materials required for nucleic acid isolation (all methods)

ltem	Source			
Equipment				
Incubators or ovens at 60°C and 90°C	MLS			
Centrifuge with plate adaptors	MLS			
Adjustable micropipettors	MLS			
Multi-channel micropipettors	MLS			
Laboratory mixer (Vortex mixer or equivalent)	MLS			
Locking lid for Autolys M Tubes	A37954			
AutoLys M TubeLifter	A37956			
AutoLys M Tube Pliers	A38261			
AutoLys M Tube Rack	A37955			
Tubes, plates, and other consumables				
AutoLys M Tubes and Caps	A38738			
Nonstick, RNase-free Microfuge Tubes (1.5 mL)	AM12450			
Nonstick, RNase-free Microfuge Tubes (2 mL)	AM12475			
Aerosol-resistant pipette tips	MLS			
Reagent reservoirs	MLS			
Reagents				
Ethanol, 200 proof (absolute)	MLS			
Isopropanol, 100%	MLS			
Nuclease-Free Water	AM9938			



Table 3 Additional materials required for manual isolation

ltem	Source		
Equipment			
Fisher Scientific™ Analog Vortex Mixer	Fisher Scientific 02-215-365		
Vortex Adapter-60	AM10014		
Accessories and tubes			
DynaMag™-2 Magnet	12321D		

Table 4 Additional materials required for automated isolation

ltem	Source			
Magnetic particle processor, one of the following:				
KingFisher [™] Flex Magnetic Particle Processor with 96 Deep-Well Head	5400630			
KingFisher™ Duo Prime Magnetic Particle Processor	5400110			
Plates and combs				
96 Deep-well plates, one of the following:				
MagMAX™ Express-96 Deep Well Plates	4388476			
96 Deep-Well Plates for KingFisher™ Flex Magnetic Particle Processor	95040450			
96-well standard plates, one of the following:				
MagMAX™ Express-96 Standard Plates	4388475			
96 Standard-Well Plates for KingFisher™ Flex Magnetic Particle Processor	97002540			
Tip comb, compatible with the magnetic particle p	rocessor used:			
KingFisher™ 96 Tip Comb for DW Magnets	97002534			
MagMAX™ Express-96 Deep Well Tip Combs	4388487			
KingFisher [™] Duo 12-Tip Comb, for Microtiter 96 Deepwell plate 97003500				
Consumables				
MicroAmp [™] Clear Adhesive Film 4306311				

If needed, download the KingFisher[™] Duo Prime or Flex program

The programs required for this protocol are not pre-installed on the KingFisher[™] Duo Prime Magnetic Particle Processor or on the KingFisher[™] Flex Magnetic Particle Processor 96DW.

- 1. On the MagMAX[™] FFPE DNA/RNA Ultra Kit product web page, scroll down to the **Product Literature** section.
- 2. Right-click on the appropriate program file(s) for your sample size to download the program to your computer:

Instrument	Sections ≤40 µm
KingFisher™ Duo Prime	A31881_DUO_lg_vol_DNA_RNA_scr
Magnetic Particle Processor	ipts
KingFisher™ Flex Magnetic	A31881_FLEX_lg_vol_DNA
Particle Processor 96DW	A31881_FLEX_lg_vol_RNA

- 3. Select Save as Target to download to your computer.
- **4.** Refer to the manufacturer's documentation for instructions for installing the program on the instrument.

Procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- When working with RNA:
 - Wear clean gloves and a clean lab coat.
 - Change gloves whenever you suspect that they are contaminated.
 - Open and close all sample tubes carefully. Avoid splashing or spraying samples.
 - Use a positive-displacement pipettor and RNase-free pipette tips.

- Clean lab benches and equipment periodically with an RNase decontamination solution, such as RNaseZap[™] Solution (Cat. No. AM9780).
- Incubation at 60°C can be extended overnight to increase DNA yields, followed by incubation at 90°C for 1 hour.
- Volumes for reagent mixes are given per sample. We recommend that you prepare master mixes for larger sample numbers. To calculate volumes for master mixes, refer to the per-well volume and add 5–10% overage.

Before you begin

Before first use of the kit

- Prepare the Wash Solutions from the concentrates:
 - Add 46 mL of isopropanol to RNA Wash Buffer Concentrate, mix, and store at room temperature.
 - Add 168 mL of ethanol to Wash Solution 2 Concentrate, mix, and store at room temperature.

Before each use of the kit

- Equilibrate the Nucleic Acid Binding Beads to room temperature.
- Pre-heat the incubators or ovens to 60°C and 90°C.
- Prepare the following solutions according to the following tables.
 Table 5 Protease solution

ReagentsVolumeProtease10 μLProtease Digestion Buffer225 μLTotal Protease Solution235 μL

Table 6 DNA Binding Buffer

Reagents	Volume
Binding Solution	250 µL
Nucleic Acid Binding Beads	20 µL
Total DNA Binding Buffer	270 µL

Table 7 RNA Binding Buffer

Reagents	Volume
Binding Solution	150 μL
Isopropanol	500 μL
Total RNA Binding Buffer	650 μL

Table 8 DNase Solution

Reagents	Volume
DNase	20 µL
DNase Buffer	10 µL
Nuclease-Free Water	70 µL
Total DNase Solution	100 µL

Table 9 RNA Rebinding Buffer

Reagents	Volume
Binding Solution	200 µL
Isopropanol	250 µL
Total RNA Rebinding Buffer	450 μL

Prepare the FFPE samples

- For curls from FFPE tissue blocks: proceed to "Prepare the curls from FFPE tissue blocks" on page 3.
- For FFPE slide-mounted sections: proceed to "Prepare samples from FFPE slides" on page 3.

Prepare the curls from FFPE tissue blocks

1	Section FFPE tissue blocks	a.	Cut sections from FFPE tissue blocks using a microtome.
÷			Note: For miRNA extraction, we recommend using sections of 10 µm or thicker.
		b.	Collect each section in an AutoLys M tube.
2	Digest with Protease	a.	Add 235 µL of the Protease Solution (see Table 5).
		b.	Note: If working with curls, they might stick straight up so make sure to submerge samples in the Protease Solution with a tip or a 1 mL syringe plunger or do a quick spin down at 3000 rpm for 1 minut prior to the addition of buffer to collapse the curl. Time may be extended. Incubate at 60°C for 1 hour or longer.
			Note: Use the AutoLys racks and place in an incubator or oven.
		c.	Incubate at 90°C for 1 hour.
			Note: For automated isolation, set up the processing plates during the incubation.
			• For isolation using KingFisher [™] Duo Prime Magnetic Particle Processor, proceed to "Set up the processing plates" on page 5.
			• For isolation using KingFisher [™] Flex Magnetic Particle Processor 96DW, proceed to "Set up the DNA processing plates" on page 6.
2	Lift the tubes	a.	Allow samples to cool down for 3–5 minutes before proceeding to lift the tubes.
J		b.	Use the Auto-plier for individual tube lifting or the Auto-lifter for multiple tube lifting of up to 24 tube
		c.	Lock the tubes in position by hand or use the locking lid.
		d.	Centrifuge at 2000 \times g for 10 minutes in a benchtop centrifuge with plate adapters.
		e.	Unlock the tubes by hand or remove the locking lid.
		f.	Use the Auto-plier or Auto-lifer to lift the inner tube for sample access.
		a.	Proceed to purification. See "Isolate DNA and RNA" on page 4
		<i>.</i> .	I FO

Prepare samples from FFPE slides

4	Scrape the samples and digest with Protease	a.	Pipet 2–4 μ L of Protease Digestion Buffer depending on the tissue size evenly across the FFPE tissue section on the slide to pre-wet the section.
			Note: You can adjust the volume of Protease Digestion Buffer if the tissue is smaller or larger.
		b.	Scrape the tissue sections in a single direction with a clean razor blade or scalpel, then collect the tissue on the slide into a cohesive mass.
		c.	Transfer the tissue mass into an AutoLys M tube with the scalpel or a pipette tip.
		d.	Add 235 µL of the Protease Solution (see Table 5).
			Note: Be sure to submerge samples in the Protease Solution with a tip or a 1 mL syringe plunger
		e.	Incubate at 60°C for 1 hour or longer.
			Note: Use the AutoLys racks and place in an incubator or oven.
		f.	Incubate at 90°C for 1 hour.
			Note: For automated isolation, set up the processing plates during the incubation.
			• For isolation using KingFisher [™] Duo Prime Magnetic Particle Processor, proceed to "Set up the processing plates" on page 5.
			• For isolation using KingFisher [™] Flex Magnetic Particle Processor 96DW, proceed to "Set up the DNA processing plates" on page 6.
5	Lift the tubes	a.	Allow samples to cool down for 3–5 minutes before proceeding to lift the tubes.
C		b.	Use the Auto-plier for individual tube lifting or the Auto-lifter for multiple tube lifting of up to 24 tube
		с.	Lock the tubes in position by hand or use the locking lid.
		d.	Centrifuge at $2000 \times g$ for 10 minutes in a benchtop centrifuge with plate adapters.
		e.	Unlock the tubes by hand or remove the locking lid.
		f.	Use the Auto-plier or Auto-lifer to lift the inner tube for sample access.
		q.	Proceed to purification. See "Isolate DNA and RNA" on page 4

Isolate DNA and RNA

- To isolate DNA and RNA manually, proceed to "Isolate DNA and RNA manually" on page 4.
- To isolate DNA and RNA using the KingFisher[™] Duo Prime Magnetic Particle Processor, proceed to "Isolate DNA and RNA using KingFisher[™] Duo Prime Magnetic Particle Processor" on page 5.
- To isolate DNA and RNA using the KingFisher[™] Flex Magnetic Particle Processor 96DW, proceed to "Isolate DNA and RNA using KingFisher[™] Flex Magnetic Particle Processor 96DW" on page 6.

Isolate DNA and RNA manually

Use microcentrifuge tubes to perform manual DNA and RNA isolations.

1	Bind the DNA to beads and	a.	Add 270 μ L of DNA Binding Buffer (see Table 6) to the sample.
	collect the RNA-containing		Note: Precipitants may form, but they do not interfere with the DNA binding.
	supernatant	b.	Shake for 5 minutes at speed 10 or 1150 rpm.
			The mixture should be chocolate brown in color.
		c.	Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are
			pelleted against the magnet.
		d.	Carefully transfer the RNA-containing supernatant with a pipette to a new tube.
			IMPORTANT! Save the RNA-containing supernatant for RNA isolation.
2	Wash DNA on the beads		Wash the beads with 400 μ L of DNA Wash Buffer.
-			Shake for 1–2 minutes at speed 9 or 1100 rpm until the mixture is thoroughly chocolate brown in color.
			Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.
		d.	Carefully discard the supernatant with a pipette.
			Repeat steps a-d.
		f.	Wash the beads with 500 μ L of Wash Solution 2.
		g.	Shake for 1 minute at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.
		h.	Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.
		i	Carefully discard the supernatant with a pipette.
		i.	Repeat steps f-i.
		k.	Shake for 1–3 minutes at speed 10 or 1150 rpm to dry the beads.
			Do not over-dry the beads. Over-dried beads results in low NA recovery yields.
			· · · · · · · · · · · · · · · · · · ·
3	Elute the DNA	a. L	Add 50 μ L of Elution Solution to the beads.
		υ. c	Shake for 5 minutes at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are
		с.	pelleted against the magnet.
			The supernatant contains the purified DNA
			The purified DNA is ready for immediate use. Store at -20° C or -80° C for long-term storage.
	Bind the RNA to beads	а	Add 20 µL of Nucleic Acid Binding Beads to the RNA-containing supernatant.
4	bind the triat to bedus	b.	Add $650 \mu\text{L}$ of RNA Binding Buffer (see Table 7) to the sample.
		c.	Shake for 5 minutes at speed 10 or 1150 rpm.
		d.	Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are
			pelleted against the magnet.
		e.	Carefully discard the supernatant with a pipette.
5	Wash RNA on the beads	a.	Wash the beads with 500 μ L of RNA Wash Buffer.
5		b.	Shake for 1 minute at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.
		с.	Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are
		لہ	pelleted against the magnet.
			Carefully discard the supernatant with a pipette. Wash the beads with 500 μ L of Wash Solution 2.
		f.	Shake for 1 minute at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.
			Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are
		-	pelleted against the magnet.
			Carefully discard the supernatant with a pipette.
		i.	Shake for 1–2 minutes at speed 10 or 1150 rpm to dry the beads.
			Do not over-dry the beads. Over-dried beads results in low NA recovery yields.
6	Treat RNA with DNase on	a.	Add 100 µL of DNase Solution (see Table 8) to the beads.
6	the beads		Shake at speed 8 or 1000 rpm for 20 minutes at 37°C or room temperature.
			Note: Shaking at 37°C increases the efficiency of the DNase digestion.
			· · · · · · · · · · · · · · · · · · ·

6	Treat RNA with DNase on the beads		Add 450 μ L of RNA Rebinding Buffer (see Table 9) to the sample. Shake for 5 minutes at speed 10 or 1150 rpm.
7	Wash the RNA on the beads after DNase treatment	a.	Place the sample on the magnetic stand for 5 minutes or until the solution clears and the beads are pelleted against the magnet.
		b.	Carefully discard the supernatant with a pipette.
		c.	Wash the beads with 500 μ L of RNA Wash Buffer.
		d.	Shake for 1 minute at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.
		e.	Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.
		f.	Carefully discard the supernatant with a pipette.
		g.	Wash the beads with 500 μ L of Wash Solution 2.
		h.	Shake for 1 minute at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.
		i.	Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.
		j.	Carefully discard the supernatant with a pipette.
		k.	Repeat steps g-j.
		ι.	Shake for 1–3 minutes at speed 10 or 1150 rpm to dry the beads.
			Do not over-dry the beads. Over-dried beads results in low RNA recovery yields.
8	Elute the RNA	a.	Add 50 μ L of Elution Solution to the beads.
0		b.	Shake for 5 minutes at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.
		c.	Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.
			The supernatant contains the purified RNA.
			The purified RNA is ready for immediate use. Store at –20°C or –80°C for long-term storage.

Isolate DNA and RNA using KingFisher[™] Duo Prime Magnetic Particle Processor

9 Set up the processing plates During the protease incubation, add processing reagents to the wells of 2 MagMAX[™] Express-96 Deep Well Plates as indicated in the following table.

Table 10 DNA plate setup

Row ID	Plate row ^[1]	Reagent	Volume per well
Sample ^[2]	A	DNA Binding Buffer (see Table 6)	270 µL
DNA Wash Buffer 1	В	DNA Wash Buffer	400 µL
DNA Wash Buffer 2	С	DNA Wash Buffer	400 µL
Wash Solution 2 - 1	D	Wash Solution 2	500 µL
Wash Solution 2 - 2	E	Wash Solution 2	500 µL
Tip Comb	F	Place a KingFisher™ Duo 12-Tip Comb	
Empty	G	Empty	
Elution	Н	Elution Solution	50 µL

^[1] Row on the MagMAX[™] Express-96 Deep Well Plate.

[2] The instrument prompts the user to add the Nucleic Acid Binding Beads and RNA Binding Buffer (see Table 7) in this order to the Sample Row after the DNA elution step.

Table 11 RNA plate setup

Row ID	Plate row ^[1]	Reagent	Volume per well
DNase ^[2]	А	DNase Solution (see Table 8)	100 µL
RNA Wash Buffer 1	В	RNA Wash Buffer	500 µL
RNA Wash Buffer 2	С	RNA Wash Buffer	500 µL
Wash Solution 2 - 1	D	Wash Solution 2	1 mL
Wash Solution 2 - 2	E	Wash Solution 2	1 mL
Empty	F	Empty	•
Empty	G	Empty	
Elution	Н	Elution Solution	50 μL

^[1] Row on the MagMAX[™] Express-96 Deep Well Plate.

[2] The instrument prompts the user to add 450 µL of RNA Rebinding Buffer (see Table 9) to the DNase Row after the DNase treatment step.

10	Bind, wash, rebind, and elute the DNA and RNA	a.	Ensure that the instrument is set up for processing with the deep well 96–well plates and select the appropriate program A31881_DUO_lg_vol_DNA_RNA_scripts on the instrument.		
		b.	At the end of the protease incubation, add 200 μ L of sample to each well in Row A of the DNA		
		с.	Start the run and load the prepared processing plates when prompted by the instrument.		
		d.	When first prompted by the instrument (after the DNA elution for RNA binding), remove the DNA plate from the instrument.		
		e.	Add 20 μ L of Nucleic Acid Binding Beads to each sample well in Row A containing the RNA supernatant.		
		f.	Add 650 μL of RNA Binding Buffer to each sample.		
		g.	Load the plate back onto the instrument, then press Start .		
		h.	When prompted next by the instrument (after DNase treatment), remove the RNA plate from the instrument.		
		i.	Add 450 μ L of RNA Rebinding Buffer (see Table 9) to each sample well in Row A.		
		j.	Load the plate back onto the instrument, then press Start .		
		k.	At the end of the run, remove the two plates from the instrument and transfer the eluted DNA (Row H of the DNA plate) and the eluted RNA (Row H of RNA plate) to two new plates and seal immediately with a new MicroAmp [™] Clear Adhesive Films.		
			IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.		
			The purified samples are ready for immediate use. Store at –20°C or –80°C for long-term storage.		
Isolate	DNA and RNA using Kir	naFis	her™ Flex Magnetic Particle Processor 96DW		

During the protease incubation, add processing reagents to the wells of MagMAX[™] Express-96 Plates as Set up the DNA processing 11 indicated in the following table. plates

Table 12 DNA plates setup

Plate ID	Plate position ^[1]	Plate type	Reagent	Volume per well
Sample Plate	1	Deep Well	DNA Binding Buffer (see Table 6)	270 µL
DNA Wash Buffer Plate 1	2	Deep Well	DNA Wash Buffer	400 µL
DNA Wash Buffer Plate 2	3	Deep Well	DNA Wash Buffer	400 µL
Wash Solution 2 Plate 1	4	Deep Well	Wash Solution 2	500 µL
Wash Solution 2 Plate 2	5	Deep Well	Wash Solution 2	500 µL
Elution Plate	6	Standard or Deep Well	Elution Solution	50 μL
Tip Comb	7	Place a Mag	AX™ Express-96 Deep Well Tip Cor	nb in a plate.

^[1] Position on the instrument

12 Bind, wash, and elute the DNA

Ensure that the instrument is set up for processing with the deep well magnetic head and select the a. A31881_FLEX_lg_vol_DNA program on the instrument.

- At the end of the protease incubation, add 200 µL of sample to each well in DNA Plate 1. b.
- Start the run and load the prepared processing plates in their positions when prompted by the с. instrument (see "Set up the DNA processing plates" on page 6).

During the run, proceed to "Set up the RNA processing plates" on page 7 to set up the RNA processing plates.

d. At the end of the DNA run, remove the Elution Plate from the instrument and seal immediately with a new MicroAmp[™] Clear Adhesive Film.

IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified DNA is ready for immediate use. Store at -20°C or -80°C for long-term storage. e. Remove the plates in positions 1–8 from the instrument.

IMPORTANT! Save the Sample Plate containing the RNA supernatant at the position 1 for RNA isolation.

13 Set up the RNA processing During the DNA isolation, add processing reagents to the wells of MagMAX[™] Express-96 Plates as indicated in the following table.

Table 13 RNA plates setup

Plate ID	Plate position ^[1]	Plate type	Reagent	Volume per well
RNA Wash Buffer Plate 1	2	Deep Well	RNA Wash Buffer	500 µL
Wash Solution 2 Plate 1	3	Deep Well	Wash Solution 2	1 mL
DNase plate	4	Deep Well	DNase Solution	100 µL
RNA Wash Buffer Plate 2	5	Deep Well	RNA Wash Buffer	500 µL
Wash Solution 2 Plate 2	6	Deep Well	Wash Solution 2	1 mL
Elution Plate	7	Standard or Deep Well	Elution Solution	50 µL
Tip Comb	8	Place a MagM	AX™ Express-96 Deep Well Tip Co	mb in a plate.

^[1] Position on the instrument

a. Ensure that the instrument is set up for processing with the deep well magnetic head and select the A31881_FLEX_lg_vol_RNA program on the instrument.

- **b.** Add 20 μ L of Nucleic Acid Binding Beads to each sample well in Plate 1.
- c. Add 650 µL of RNA Binding Buffer (see Table 7) to each sample well.
- **d**. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see "Set up the RNA processing plates" on page 7).
- e. When prompted by the instrument (after the DNase treatment), remove the DNase plate from the instrument.
- f. Add 450 µL of RNA Rebinding Buffer (see Table 9) to each sample well.
- g. Load the plate back onto the instrument, and press Start.
- h. At the end of the run, remove the Elution Plate from the instrument and seal immediately with a new MicroAmp[™] Clear Adhesive Film.

IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified RNA is ready for immediate use. Store at -20°C or -80°C for long-term storage.

Limited product warranty

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Revision	Date	Description
A.0	26 February 2018	New document

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14 Bind, wash, rebind, and elute the RNA