

MagMAX™ FFPE DNA/RNA Ultra Kit

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

Catalog Number A31881

Pub. No. MAN0017540 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™ magnetic-bead 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 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 sequential DNA and RNA isolation, or DNA isolation, or TNA isolation only, see *MagMAX™ FFPE DNA/RNA Ultra Kit User Guide (sequential DNA/RNA isolation)* (Pub. No. MAN0017541), or *MagMAX™ FFPE DNA/RNA Ultra Kit User Guide (DNA isolation only)* (Pub. No. MAN0017539), 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 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 ^[3]	38.5 mL	15°C to 30°C
Wash Solution 2 Concentrate	210 mL ^[4]	
Elution Solution	5 mL	
RNA Wash Buffer Concentrate	115 mL ^[4]	–25°C to –15°C
DNase	1.95 mL	
DNase buffer	960 µL	

^[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] Not used in RNA workflow.

^[4] Final volume; see “Isolate 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)

Item	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

Item	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

Item	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 processor 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 Magnetic Particle Processor	A31881_DUO_large_vol_RNA_only
KingFisher™ Flex Magnetic Particle Processor 96DW	A31881_FLEX_large_vol_RNA_only

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).
- Incubations at 60°C and 90°C during the protease digestion can be shortened down to 15 minutes. However, longer incubation times result in higher RNA yields.
- 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

Reagents	Volume
Protease	10 µL
Protease Digestion Buffer	225 µL
Total Protease Solution	235 µL

Table 6 RNA Binding Buffer

Reagents	Volume
Binding Solution	400 µL
Isopropanol	500 µL
Total RNA Binding Buffer	900 µL

Table 7 DNase Solution

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

Table 8 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	<p>a. Cut sections from FFPE tissue blocks using a microtome.</p> <p>Note: For miRNA extraction, we recommend using sections of 10 µm or thicker.</p> <p>b. Collect each section in an AutoLys M tube.</p>
2	Digest with Protease	<p>a. Add 235 µL of the Protease Solution (see Table 5).</p> <p>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 minute prior to the addition of buffer to collapse the curl. Time may be extended.</p> <p>b. Incubate at 60°C for up to 1 hour.</p> <p>Note: Use the AutoLys racks and place in an incubator or oven.</p> <p>c. Incubate at 90°C for up to 1 hour.</p> <p>Note: For automated isolation, set up the processing plates during the incubation.</p> <ul style="list-style-type: none">• For isolation using KingFisher™ Duo Prime Magnetic Particle Processor, proceed to “Set up the processing plate” on page 5.• For isolation using KingFisher™ Flex Magnetic Particle Processor 96DW, proceed to “Set up the RNA processing plates” on page 5.
3	Lift the tubes	<p>a. Allow samples to cool down for 3–5 minutes before proceeding to lift the tubes.</p> <p>b. Use the Auto-plier for individual tube lifting or the Auto-lifter for multiple tube lifting of up to 24 tubes.</p> <p>c. Lock the tubes in position by hand or use the locking lid.</p> <p>d. Centrifuge at 2000 × g for 10 minutes in a benchtop centrifuge with plate adapters.</p> <p>e. Unlock the tubes by hand or remove the locking lid.</p> <p>f. Use the Auto-plier or Auto-lifter to lift the inner tube for sample access.</p> <p>g. Proceed to purification. See “Isolate RNA” on page 4</p>

Prepare samples from FFPE slides

4	Scrape the samples and digest with Protease	<p>a. Pipet 2–4 µL of Protease Digestion Buffer depending on the tissue size evenly across the FFPE tissue section on the slide to pre-wet the section.</p> <p>Note: You can adjust the volume of Protease Digestion Buffer if the tissue is smaller or larger.</p> <p>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.</p> <p>c. Transfer the tissue mass into an AutoLys M tube with the scalpel or a pipette tip.</p> <p>d. Add 235 µL of the Protease Solution (see Table 5).</p> <p>Note: Be sure to submerge samples in the Protease Solution with a tip or a 1 mL syringe plunger</p> <p>e. Incubate at 60°C for up to 1 hour.</p> <p>Note: Use the AutoLys racks and place in an incubator or oven.</p> <p>f. Incubate at 90°C for up to 1 hour.</p> <p>Note: For automated isolation, set up the processing plates during the incubation.</p> <ul style="list-style-type: none">• For isolation using KingFisher™ Duo Prime Magnetic Particle Processor, proceed to “Set up the processing plate” on page 5.• For isolation using KingFisher™ Flex Magnetic Particle Processor 96DW, proceed to “Set up the RNA processing plates” on page 5.
5	Lift the tubes	<p>a. Allow samples to cool down for 3–5 minutes before proceeding to lift the tubes.</p> <p>b. Use the Auto-plier for individual tube lifting or the Auto-lifter for multiple tube lifting of up to 24 tubes.</p> <p>c. Lock the tubes in position by hand or use the locking lid.</p> <p>d. Centrifuge at 2000 × g for 10 minutes in a benchtop centrifuge with plate adapters.</p> <p>e. Unlock the tubes by hand or remove the locking lid.</p> <p>f. Use the Auto-plier or Auto-lifter to lift the inner tube for sample access.</p> <p>g. Proceed to purification. See “Isolate RNA” on page 4</p>

Isolate RNA

- To isolate RNA manually, proceed to “Isolate RNA manually” on page 4.
- To isolate RNA using the KingFisher™ Duo Prime Magnetic Particle Processor, proceed to “Isolate RNA using KingFisher™ Duo Prime Magnetic Particle Processor” on page 5.
- To isolate RNA using the KingFisher™ Flex Magnetic Particle Processor 96DW, proceed to “Isolate RNA using KingFisher™ Flex Magnetic Particle Processor 96DW” on page 5.

Isolate RNA manually

Use microcentrifuge tubes to perform manual RNA isolations.

1	Bind the RNA to the beads	<ol style="list-style-type: none">After the Protease digestion is complete, add 20 µL of Nucleic Acid Binding Beads to the samples.Add 900 µL of RNA Binding Buffer (see Table 6) to the sample.Shake for 5 minutes at speed 10 or 1150 rpm.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.
2	Wash RNA on the beads	<ol style="list-style-type: none">Wash the beads with 500 µL of RNA Wash Buffer.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.Wash the beads with 500 µL of Wash Solution 2.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.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	Treat RNA with DNase on the beads	<ol style="list-style-type: none">Add 100 µL of DNase Solution (see Table 7) to 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.Add 450 µL of RNA Rebinding Buffer (see Table 8) to the sample.Shake for 5 minutes at speed 10 or 1150 rpm.
4	Wash the RNA on the beads after DNase treatment	<ol style="list-style-type: none">Place the sample on the magnetic stand for 5 minutes or until the solution clears and the beads are pelleted against the magnet.Carefully discard the supernatant with a pipette.Wash the beads with 500 µL of RNA Wash Buffer.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.Wash the beads with 500 µL of Wash Solution 2.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.Repeat steps g-j.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.
5	Elute the RNA	<ol style="list-style-type: none">Add 50 µL of Elution Solution to the beads.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 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 RNA using KingFisher™ Duo Prime Magnetic Particle Processor

- 6** Set up the processing plate During the protease incubation, add processing reagents to the wells of a MagMAX™ Express-96 Deep Well Plate as indicated in the following table.

Table 9 RNA plate setup

Row ID	Plate row ^[1]	Reagent	Volume per well
DNase ^[2]	A	DNase Solution (see Table 7)	100 µL
RNA Wash Buffer 1	B	RNA Wash Buffer	500 µL
RNA Wash Buffer 2	C	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
Tip Comb	F	Place a KingFisher™ Duo 12-Tip Comb	
Sample	G	RNA Binding Buffer (see Table 6)	450 µL
Elution	H	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 8) to the DNase Row after the DNase treatment step.

- 7** Bind, wash, rebind, and elute the RNA
- Ensure that the instrument is set up for processing with the deep well 96–well plates and select the appropriate program **A31881_DUO_large_vol_RNA_only** on the instrument.
 - At the end of the protease incubation, add 200 µL of sample to each well in Row G of the RNA plate.
 - Add 20 µL of Nucleic Acid Binding Beads to each sample well in Row G.
 - Start the run and load the prepared processing plate when prompted by the instrument.
 - When prompted next by the instrument (after DNase treatment), remove the RNA plate from the instrument.
 - Add 450 µL of RNA Rebinding Buffer (see Table 8) to each sample well in Row A.
 - Load the plate back onto the instrument, then press **Start**.
 - At the end of the run, remove the Elution Plate from the instrument and transfer the eluted RNA (Row H of RNA plate) to a new plate 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.

Isolate RNA using KingFisher™ Flex Magnetic Particle Processor 96DW

- 8** Set up the RNA processing plates During the protease incubation, add processing reagents to the wells of MagMAX™ Express-96 Plates as indicated in the following table.

Table 10 RNA plates setup

Plate ID	Plate position ^[1]	Plate type	Reagent	Volume per well
Sample	1	Deep Well	RNA Binding Buffer (see Table 6)	900 µL
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 ^[2]	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 MagMAX™ Express-96 Deep Well Tip Comb in a plate.		

^[1] Position on the instrument

^[2] The instrument prompts the user to add 450 µL of RNA Rebinding Buffer to the DNase plate after the DNase treatment step.

- 9** Bind, wash, rebind, and elute the RNA
- Ensure that the instrument is set up for processing with the deep well magnetic head and select the **A31881_FLEX_large_vol_RNA_only** program on the instrument.
 - At the end of the protease incubation, add 200 µL of sample to each well in Plate 1.
 - Add 20 µL of Nucleic Acid Binding Beads to each sample well in Plate 1.
 - 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 5).
 - When prompted by the instrument (after the DNase treatment), remove the DNase plate from the instrument.
 - Add 450 µL of RNA Rebinding Buffer (see Table 8) to each sample well.

9 Bind, wash, rebind,
and elute the RNA
(continued)

- 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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