MagMAX™ FFPE DNA/RNA Ultra Kit

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

Catalog Number A31881

Pub. No. MAN0015877 Rev. D.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 or RNA only. 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 applications, 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 using AutoLys M tubes for sequential DNA and RNA isolation, or DNA isolation, or RNA 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 (RNA isolation only)* (Pub. No. MAN0017540), or *MagMAX™ FFPE DNA/RNA Ultra Kit User Guide (TNA isolation only)* (Pub. No. MAN0017538), respectively.

This guide describes sequential isolation of DNA and RNA from FFPE tissue blocks or FFPE slides. Four optimized methods 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).
- KingFisher™ Apex Purification System with 96 Deep-Well Head (96 DW: 96-well deep well setting) or 96 Combi Head (96 Combi: 96-well deep well setting).

These methods are provided for sections or curls both up to 40 µm and greater than 40 µm.

For DNA or RNA isolation only, see *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.

Contents and storage

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

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[1]	38.5 mL	15°C to 30°C
Wash Solution 2 Concentrate	210 mL[3]	
Elution Solution	5 mL	
RNA Wash Buffer Concentrate	115 mL[3]	
DNase	1.95 mL	–25°C to –15°C
DNase buffer	960 µL	

[1] For processing sections >40 µm, additional reagents are required. 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 "Before first use of the kit" on page 2.

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

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

Table 2 Materials required for DNA and RNA isolation (all methods)

Item	Source
Equipment	
Heat blocks, water baths, or incubators at 50°C, 55°C, and 90°C	MLS
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Laboratory mixer (vortex mixer or equivalent)	MLS
(Optional) Centrifugal vacuum concentrator	MLS
Tubes, plates, and other consumables	
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450
Nonstick, RNase-Free Microfuge Tubes, 2.0 mL	AM12475
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS
Reagents	
Citrisolv Clearing Agent, or equivalent (xylene, other solvent)	Fisher Scientific 22-143-975
Ethanol, 200 proof (absolute)	MLS
Isopropanol, 100%	MLS
Nuclease-free water	AM9938

Table 3 Additional materials required for manual isolation

Item	Source
Equipment	
Fisherbrand™ Analog Vortex Mixer	Fisher Scientific 02-215-414
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
KingFisher™ Apex Purification System with 96 DW Head	5400930
Plates and combs	
96 deep-well plates, one of the following:	
KingFisher™ 96 Deep-Well Plates, v-bottom, polypropylene	95040450
KingFisher™ 96 Deep-Well Plates, barcoded	95040450B
96-well standard plates:	
KingFisher™ 96 KF microplates	97002540
Tip comb, compatible with the magnetic particle processor used:	
KingFisher™ 96 tip comb for DW magnets	97002534
KingFisher™ Duo 12-tip Comb, for 96 deep-well plate	97003500
KingFisher™ Apex 96 Combi tip comb	97002570
Consumables	
MicroAmp™ Clear Adhesive Film	4306311

If needed, download the instrument protocol

Download, then install the KingFisher™ Duo Prime or KingFisher™ Flex protocol

- The appropriate protocol for the kit must be downloaded and installed on the instrument before use.

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 file(s) according to your sample size, then select **Save as Target** to download.

Instrument	Sections ≤40 µm	Sections >40 µm
KingFisher™ Duo Prime	**A31881_DUO_std_DNA_RNA_scripts**	**A31881_DUO_lg_vol_DNA_RNA_scripts**
KingFisher™ Flex	**A31881_FLEX_std_DNA_RNA_scripts**	**A31881_FLEX_lg_vol_DNA_RNA_scripts**

3. See the instrument user guide for instructions for installing the protocol on the instrument.

Download the KingFisher™ Apex protocol from the Thermo Fisher™ Connect Platform

1. Sign in to your Connect account and go to **https://apps.thermofisher.com/apps/kingfisher/#/protocol-library**.

   See your instrument user guide for instructions to link the instrument to your Connect account.
2. Select **(InstrumentConnect)** from the left navigation strip.
3. Select the appropriate protocol(s) according to your sample size, then click **(Transfer to instrument)**.

Instrument	Sections ≤40 µm	Sections >40 µm
KingFisher™ Apex with 96 DW Head	**MagMAX_FFPE_DNA_v1** **MagMAX_FFPE_RNA_v1**	**MagMAX_FFPE_lg_vol_DNA_v1** **MagMAX_FFPE_lg_vol_RNA_v1**

4. Select the instrument where you want to transfer the protocol, then click **Transfer**.
5. See the instrument user guide for instructions for installing the protocol 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 RNase*Zap*™ Solution (Cat. no. AM9780).
- 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.
- Incubation at 55°C can be extended overnight to increase DNA yields followed by the 90°C for 1 hour.

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 heat blocks, water baths, or incubators to 50°C, 55°C, and 90°C.
- Prepare the following solutions according to the following tables.

Table 5 Protease solution

Reagents	Sections ≤40 µm	Sections >40 µm
Protease	10 µL	10 µL
Protease Digestion Buffer	100 µL	200 µL
Total Protease Solution	110 µL	210 µL

Table 6 DNA Binding Buffer

Reagents	Sections ≤40 µm	Sections >40 µm
Binding Solution	135 µL	250 µL
Nucleic Acid Binding Beads	20 µL	20 µL
Total DNA Binding Buffer	155 µL	270 µL

Table 7 RNA Binding Buffer

Reagents	Sections ≤40 µm	Sections >40 µm
Binding Solution	100 µL	130 µL
Isopropanol	300 µL	450 µL
Total RNA Binding Buffer	400 µL	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 4.

Prepare the curls from FFPE tissue blocks

1 Section FFPE tissue blocks

1.1. Cut sections from FFPE tissue blocks using a microtome.

Note: For miRNA extraction, we recommend using sections of 10 µm or thicker.

1.2. Collect each section in a 1.5-mL microcentrifuge tube.

2 Remove paraffin from the sections

2.1. Preheat a heating block (with lid) or incubator at 50°C.

2.2. Add 1 mL of Citrisolv Clearing Agent, or equivalent (xylene, other solvent) to the section, and vortex briefly.

2.3. Centrifuge briefly to ensure that all the tissue is submerged in the solvent.

2.4. Heat the sample for 3 minutes at 50°C to melt the paraffin.

2.5. Centrifuge the sample at maximum speed for 2 minutes to pellet the tissue.

- If the sample does not form a tight pellet, centrifuge again for 2 minutes.
- If a tight pellet still does not form, proceed with caution to the next step.

2.6. Remove and discard the solvent.

Note: If the pellet is loose, leave 50–100 µL of solvent in the tube to avoid removing any tissue pieces.

The tissue is usually clear and can be difficult to see.

3 Wash twice with ethanol

3.1. Add 1 mL of 100% ethanol to the tissue pellet and vortex.

The tissue should turn opaque.

3.2. Centrifuge the sample at maximum speed for 2 minutes.

3.3. Remove and discard as much ethanol as possible without disturbing the pellet.

3.4. Perform a second ethanol wash by repeating step 3a through step 3c to ensure complete solvent removal.

IMPORTANT! Omit the second wash when working with small samples as excess washing can result in sample loss.

4 Dry the tissue pellet

Times will vary depending on how much ethanol is present.

Dry the pellet using one of the following methods:

- Use a centrifugal vacuum concentrator with one of the following settings.

Temperature	Time
40–45°C (medium heat)	<20 minutes
37–40°C (low heat)	20–40 minutes

- Air dry at room temperature for 15–45 minutes.

STOPPING POINT *(Optional)* The dried samples can be stored at room temperature up to 72 hours.

5 Digest with Protease

5.1. Add Protease Solution (see Table 5) to each sample according to the following table.

Sections	Protease Solution volume
≤40 µm	110 µL
>40 µm	210 µL

5.2. Gently flick the tube to mix and to immerse the tissue.

If the tissue sticks to the sides of the tube, use a pipet tip to push the tissue into the solution or centrifuge briefly to immerse the tissue in the solution.

5.3. Incubate at 55°C for 1 hour or longer, then centrifuge briefly to collect any condensation droplets.

Note: If you are using an incubator, use a 4-way microtube rack to allow homogeneous incubation of the samples.

5.4. Incubate at 90°C for 1 hour, then centrifuge briefly to collect any condensation droplets.

Note: Ensure that tubes are tightly capped. Tube caps may pop open during the incubation.

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 6.

5 Digest with Protease *(continued)*

- For isolation using KingFisher™ Flex Magnetic Particle Processor 96DW, proceed to "Set up the DNA processing plates" on page 7.
- For isolation using KingFisher™ Apex Purification System with 96 DW Head, proceed to "Set up the DNA processing plates" on page 9.

Allow samples to cool down before proceeding to next step.

Prepare samples from FFPE slides

1 Remove paraffin from the sections

1.1. Submerge the slides in Citrisolv Clearing Agent, or equivalent (xylene, other solvent) for 5 minutes.
1.2. Remove the slides, then drain the excess solvent by tilting the slide holder.
1.3. Submerge the slides in 100% ethanol for 5 minutes.
1.4. Remove the slides, then drain the excess ethanol by tilting the slide holder.
1.5. Air dry the slides for 15 minutes.

2 Digest with Protease

2.1. 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.

Note: You can adjust the volume of Protease Digestion Buffer if the tissue is smaller or larger.

2.2. 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.
2.3. Transfer the tissue mass into a sterile 1.5-mL tube with the scalpel or a pipette tip.
2.4. Add Protease Solution (see Table 5) to each sample according to the following table.

Sections	Protease Solution volume
≤40 µm	110 µL
>40 µm	210 µL

2.5. Gently flick the tube to mix and to immerse the tissue.

If the tissue sticks to the sides of the tube, use a pipette tip to push the tissue into the solution or centrifuge briefly to immerse the tissue in the solution.

2.6. Incubate at 55°C for 1 hour or longer, then centrifuge briefly to collect any condensation droplets.

Note: If you are using an incubator, use a 4-way microtube rack to allow homogeneous incubation of the samples.

2.7. Incubate at 90°C for 1 hour, then centrifuge briefly to collect any condensation droplets.

Note: Ensure that tubes are tightly capped. Tube caps may pop open during the incubation.

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 6.
- For isolation using KingFisher™ Flex Magnetic Particle Processor 96DW, proceed to "Set up the DNA processing plates" on page 7.
- For isolation using KingFisher™ Apex Purification System with 96 DW Head, proceed to "Set up the DNA processing plates" on page 9.

Allow samples to cool down before proceeding to next step.

Isolate DNA and RNA sequentially

- 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 6.
- 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 7.
- To isolate DNA and RNA using the KingFisher™ Apex Purification System with 96 DW Head, proceed to "Isolate DNA and RNA using KingFisher™ Apex Purification System with 96 DW Head" on page 9.

Isolate DNA and RNA manually

Use microcentrifuge tubes to perform manual DNA and RNA isolations.

1 Bind DNA to the beads and collect the RNA-containing supernatant

1.1. Add DNA Binding Buffer (see Table 6) to the digested sample according to the following table.

Sections	DNA Binding Buffer volume
≤40 µm	155 µL
>40 µm	270 µL

Note: Precipitants may form, but they don't interfere with the DNA binding. Proceed directly to the next step.

1 Bind DNA to the beads and collect the RNA-containing supernatant *(continued)*

1.2. Mix for 5 minutes according to the following table.

Sections	Mixing method
≤40 µm	Shake at speed 8 or 1000 rpm
>40 µm	Shake at speed 10 or 1150 rpm

The mixture should be chocolate brown in color.

1.3. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.

1.4. Carefully transfer the RNA-containing supernatant with a pipette to a new tube.

IMPORTANT! Save the RNA-containing supernatant for RNA isolation.

2 Wash the DNA on the beads

2.1. Wash the beads with DNA Wash Buffer according to the following table.

Sections	DNA Wash Buffer volume
≤40 µm	200 µL
>40 µm	400 µL

2.2. Shake for 1–2 minutes at speed 9 or 1100 rpm until the mixture is thoroughly chocolate brown in color.

2.3. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.

2.4. Carefully discard the supernatant with a pipette.

2.5. Repeat step 2a-step 2d.

2.6. Wash the beads with Wash Solution 2 according to the following table.

Sections	Wash Solution 2 volume
≤40 µm	200 µL
>40 µm	500 µL

2.7. Shake for 1 minute at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.

2.8. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.

2.9. Carefully discard the supernatant with a pipette.

2.10. Repeat step 2f-step 2i.

2.11. 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 DNA recovery yields.

3 Elute the DNA

3.1. Add 50 µL of Elution Solution to the beads.

3.2. Shake for 5 minutes at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.

3.3. 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 DNA

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

4 Bind the RNA to beads

4.1. Add 20 µL of Nucleic Acid Binding Beads to the RNA-containing supernatant.

4.2. Add RNA Binding Buffer (see Table 7) to the sample according to the following table.

Sections	RNA Binding Buffer volume
≤40 µm	400 µL
>40 µm	580 µL

4.3. Shake for 5 minutes at speed 10 or 1150 rpm.

4.4. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.

4.5. Carefully discard the supernatant with a pipette.

5 Wash RNA on the beads

5.1. Wash the beads with 500 µL of RNA Wash Buffer.

5.2. Shake for 1 minute at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.

5.3. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.

5.4. Carefully discard the supernatant with a pipette.

5.5. Wash the beads with 500 µL of Wash Solution 2.

5.6. Shake for 1 minute at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.

5.7. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.

5.8. Carefully discard the supernatant with a pipette.

5.9. 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 RNA recovery yields.

6 Treat RNA with DNase on the beads

6.1. Add 100 µL of DNase Solution (see Table 8) to the beads.
6.2. 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.

6.3. Add 450 µL of RNA Rebinding Buffer (see Table 9) to the sample.
6.4. Shake for 5 minutes at speed 10 or 1150 rpm.

7 Wash the RNA on the beads after DNase treatment

7.1. Place the sample on the magnetic stand for 5 minutes or until the solution clears and the beads are pelleted against the magnet.
7.2. Carefully discard the supernatant with a pipette.
7.3. Wash the beads with 500 µL of RNA Wash Buffer.
7.4. Shake for 1 minute at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.
7.5. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.
7.6. Carefully discard the supernatant with a pipette.
7.7. Wash the beads with 500 µL of Wash Solution 2.
7.8. Shake for 1 minute at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.
7.9. Place the sample on the magnetic stand for 2 minutes or until the solution clears and the beads are pelleted against the magnet.
7.10. Carefully discard the supernatant with a pipette.
7.11. Repeat step 7g-step 7j.
7.12. 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

8.1. Add 50 µL of Elution Solution to the beads.
8.2. Shake for 5 minutes at speed 10 or 1150 rpm until the mixture is thoroughly chocolate brown in color.
8.3. 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

1 Set up the processing plates

During the protease incubation, add processing reagents to the wells of 2 KingFisher™ 96 Deep-Well Plates as indicated in the following tables.

Table 10 DNA plate setup

Row ID	Plate row[1]	Reagent	Volume per well	
			Sections ≤40 µm	Sections >40 µm
Sample[2]	A	DNA Binding Buffer (see Table 6)	155 µL	270 µL
DNA Wash Buffer 1	B	DNA Wash Buffer	200 µL	400 µL
DNA Wash Buffer 2	C	DNA Wash Buffer	200 µL	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.		
	G	Empty		
Elution	H	Elution Solution	50 µL	

[1] Row on the KingFisher™ 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]	A	DNase Solution (see Table 8)	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
	F	Empty	
	G	Empty	
Elution	H	Elution Solution	50 µL

[1] Row on the KingFisher™ 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.

2 Bind, wash, rebind, and elute DNA and RNA

2.1. Ensure that the instrument is set up for processing with the deep well 96–well plates and select the appropriate program on the instrument.

- **A31881_DUO_std_DNA_RNA_scripts** for sections ≤40 µm.
- **A31881_DUO_lg_vol_DNA_RNA_scripts** for sections >40 µm.

2.2. At the end of the protease incubation, add the samples to Row A of the DNA plate according to the following table.

Sections	Sample volume
≤40 µm	100 µL
>40 µm	200 µL

2.3. Start the run and load the prepared processing plate when prompted by the instrument (see Table 10 and Table 11).

2.4. When first prompted by the instrument (after the DNA elution for RNA binding):

a. Remove the DNA plate from the instrument.

b. Add 20 µL of Nucleic Acid Binding Beads to each sample well in Row A containing the RNA supernatant.

c. Add RNA Binding Buffer (see Table 7) to each sample according to the following table.

Sections	RNA Binding Buffer volume
≤40 µm	400 µL
>40 µm	580 µL

d. Load the plate back onto the instrument, and press **Start**.

2.5. When prompted next by the instrument (after the DNase treatment):

a. Remove the RNA plate from the instrument.

b. Add 450 µL of RNA Rebinding Buffer (see Table 9) to each sample well in Row A.

c. Load the plate back onto the instrument, and press **Start**.

2.6. At the end of the run, remove the two plates from the instrument and transfer the eluted DNA (Row H of DNA plate) and the eluted RNA (Row H of RNA plate) to two new plates and seal immediately with 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 KingFisher™ Flex Magnetic Particle Processor 96DW

1 Set up the DNA processing plates

During the protease incubation, add processing reagents to the wells of KingFisher™ 96 Deep-Well Plates as indicated in the following table.

Table 12 DNA plates setup

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well	
				Sections ≤40 µm	Sections > 40 µm
Sample Plate	1	Deep Well	DNA Binding Buffer (see Table 6)	155 µL	270 µL
DNA Wash Buffer Plate 1	2	Deep Well	DNA Wash Buffer	200 µL	400 µL
DNA Wash Buffer Plate 2	3	Deep Well	DNA Wash Buffer	200 µL	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 KingFisher™ 96 tip comb for DW magnets in a plate.			

[1] Position on the instrument.

2 Bind, wash, and elute the DNA

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

- **A31881_FLEX_std_DNA** for sections ≤40 µm.
- **A31881_FLEX_lg_vol_DNA** for sections >40 µm.

2 Bind, wash, and elute the DNA *(continued)*

2.2. At the end of the protease incubation, add the samples to the DNA Sample plate according to the following table.

Sections	Sample volume
≤40 µm	100 µL
>40 µL	200 µL

2.3. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 12).

During the run, proceed to "Set up the RNA processing plates" on page 8 to set up the RNA processing plates.

2.4. 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.

Remove the plates in positions 1-8 from the instruments.

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

3 Set up the RNA processing plates

During the DNA isolation, add processing reagents to the wells of KingFisher™ 96 Deep-Well 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 (see Table 8)	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 KingFisher™ 96 tip comb for DW magnets in a plate.		

[1] Position on the instrument.

4 Bind, wash, rebind, and elute the RNA

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

- **A31881_FLEX_std_RNA** for sections ≤40 µm.
- **A31881_FLEX_lg_vol_RNA** for sections >40 µm.

4.2. Add 20 µL of Nucleic Acid Binding Beads.

4.3. Add RNA Binding Buffer (see Table 7) to each well of the Sample Plate containing the RNA supernatant according to the following table.

Sections	RNA Binding Buffer
≤40 µm	400 µL
>40 µm	650 µL

4.4. 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 8).

4.5. When prompted by the instrument (after the DNase treatment):

a. Remove the DNase plate from the instrument.

b. Add 450 µL of RNA Rebinding Buffer (see Table 9) to each sample well.

c. Load the plate back onto the instrument, and press **Start**.

4.6. 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.

1 Set up the DNA processing plates

During the protease incubation, add processing reagents to the wells of KingFisher™ 96 Deep-Well Plates (or barcoded KingFisher™ 96 Deep-Well Plates) as indicated in the following table.

Table 14 DNA plates setup

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well	
				Sections ≤40 µm	Sections > 40 µm
Sample Plate	2	Deep Well	DNA Binding Buffer (see Table 6)	155 µL	270 µL
DNA Wash Buffer Plate 1	3	Deep Well	DNA Wash Buffer	200 µL	400 µL
DNA Wash Buffer Plate 2	4	Deep Well	DNA Wash Buffer	200 µL	400 µL
Wash Solution 2 Plate 1	5	Deep Well	Wash Solution 2	500 µL	
Wash Solution 2 Plate 2	6	Deep Well	Wash Solution 2	500 µL	
Elution Plate	7	Standard or Deep Well	Elution Solution	50 µL	
Tip Comb	1	Place a KingFisher™ 96 tip comb for DW magnets in a plate.			

[1] Position on the instrument.

2 Bind, wash, and elute the DNA

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

- **MagMAX_FFPE_DNA_v1** for sections ≤40 µm.
- **MagMAX_FFPE_lg_vol_DNA_v1** for sections >40 µm.

2.2. At the end of the protease incubation, add the samples to the DNA Sample plate according to the following table.

Sections	Sample volume
≤40 µm	100 µL
>40 µL	200 µL

2.3. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 12).

During the run, proceed to “Set up the RNA processing plates” on page 8 to set up the RNA processing plates.

2.4. 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.

Remove the plates in positions 1-8 from the instruments.

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

3 Set up the RNA processing plates

During the DNA isolation, add processing reagents to the wells of KingFisher™ 96 Deep-Well Plates (or equivalent) as indicated in the following table.

Table 15 RNA plates setup

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
RNA Wash Buffer Plate 1	3	Deep Well	RNA Wash Buffer	500 µL
Wash Solution 2 Plate 1	4	Deep Well	Wash Solution 2	1 mL
DNase Plate	5	Deep Well	DNase Solution (see Table 8)	100 µL
RNA Wash Buffer Plate 2	6	Deep Well	RNA Wash Buffer	500 µL
Wash Solution 2 Plate 2	7	Deep Well	Wash Solution 2	1 mL
Elution Plate	8	Standard or Deep Well	Elution Solution	50 µL
Tip Comb	1	Place a KingFisher™ 96 tip comb for DW magnets in a plate.		

[1] Position on the instrument

4 Bind, wash, rebind, and elute the RNA

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

- **MagMAX_FFPE_RNA_v1** for sections ≤40 µm.
- **MagMAX_FFPE_lg_vol_RNA_v1** for sections >40 µm.

4.2. Add 20 µL of Nucleic Acid Binding Beads.

4.3. Add RNA Binding Buffer (see Table 7) to each well of the Sample Plate containing the RNA supernatant according to the following table.

Sections	RNA Binding Buffer
≤40 µm	400 µL
>40 µm	650 µL

4.4. 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 8).

4.5. When prompted by the instrument (after the DNase treatment):

a. Remove the DNase plate from the instrument.

b. Add 450 µL of RNA Rebinding Buffer (see Table 9) to each sample well.

c. Load the plate back onto the instrument, and press **Start**.

4.6. 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
D.0	19 April 2021	Addition of protocol for KingFisher™ Apex Purification System with 96DW Head.
C.0	26 February 2018	Addition of publication titles and numbers for user guides using AutoLys M tubes.
B.0	14 February 2017	Clarification of the program names for the KingFisher™ Flex Magnetic Particle Processor
A.0	12 July 2016	New document