MagMAX™ FFPE Total Nucleic Acid Isolation Kit

USER GUIDE

Automated or manual isolation of DNA or RNA from FFPE samples

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About this guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Revision history

Revision	Date	Description
C	December 2015	Correction of the total volume of RNase Solution
B	June 2015	• Addition of automated protocols for KingFisher™ Duo Prime Magnetic Particle Processor and KingFisher™ Flex Magnetic Particle Processor 96DW • Updates to style, format, and legal content according to corporate guidelines
A	March 2011	New document

1 Product information

Product description

The MagMAX™ FFPE Total Nucleic Acid Isolation Kit is designed for isolation of RNA and DNA from formaldehyde- or paraformaldehyde- fixed, paraffin-embedded (FFPE) tissues. The kit uses MagMAX™ magnetic-bead technology, ensuring reproducible recovery of high-quality nucleic acid suitable for use with a broad range of applications, including microarray analysis, quantitative real-time RT-PCR, sequencing, and mutation screening, without the need for a deparaffinization step.

This protocol describes isolation of nucleic acid from up to two 10-µm sections of tissue in a plate format that is optimized for one of the following methods:

- Manual
- Automated: MagMAX™ Express-96 Deep Well Magnetic Particle Processor
- Automated: KingFisher™ Flex Magnetic Particle Processor with 96 Deep Well Head (DW96; 96-well deep well setting)
- Automated: KingFisher™ Duo Prime Magnetic Particle Processor (12-well deep well setting).

Kit contents and storage

Table 1 MagMAX™ FFPE Total Nucleic Acid Isolation Kit (Cat. no. 4463365, 96 reactions)

Contents	Amount	Storage
Box 1 of 2		
Nucleic Acid Binding Beads[1]	2 mL	2°C to 8°C
Digestion Buffer	15 mL	15°C to 30°C
RNA Digestion Additive	3 mL	
DNA Digestion Additive	3 mL	
DNase Buffer	20 mL	
Wash Solution 1 Concentrate[2]	48 mL	
Wash Solution 2 Concentrate[2]	60 mL	
Elution Buffer	7 mL	
Nuclease-Free Water	18 mL	

Contents	Amount	Storage
Processing Plate	1	15°C to 30°C
Elution Plates	2	
Box 2 of 2		
Protease	400 µL	–25°C to –15°C
DNase	500 µL	
RNase A	120 µL	

[1] Do not freeze the RNA Binding Beads.
[2] Final volume; see "Before first use: prepare Wash Solutions" on page 12.

Materials required but not supplied

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

Item	Source
Magnetic particle processor, one of the following:	
KingFisher™ Flex Magnetic Particle Processor with 96 Deep Well Head[1]	Cat. no. 5400630
MagMAX™ Express-96 Deep Well Magnetic Particle Processor	—[2]
KingFisher™ Duo Prime Magnetic Particle Processor[1]	Cat. no. 5400110
Other equipment	
Thermo Scientific™ Compact Digital Microplate Shaker	Fisher Scientific Cat. no. 11-676-337
Fisher Scientific™ Analog Vortex Mixer	Fisher Scientific Cat. no. 02-215-365
Heating blocks, water baths, or incubators at 50°C, 60°C, and 80°C	MLS
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Magnetic Stand-96	Cat. no. AM10027
(Optional) Centrifuge capable of centrifuging 96-well plates	MLS

Item	Source
Plates and combs[3]	
Deep well plates, one of the following:	
MagMAX™ Express-96 Deep Well Plates	Cat. no. 4388476
KingFisher™ Flex Microtiter Deepwell 96 Plate, Sterile	Thermo Scientific Cat. no. 95040460
Standard well plates, one of the following:	
MagMAX™ Express-96 Standard Plates	Cat. no. 4388475
KingFisher™ 96 KF Microplate	Cat. no. 97002540
Tip comb, compatible with the magnetic particle processor used:	
MagMAX™ Express-96 Deep Well Tip Combs	Cat. no. 4388487
KingFisher™ 96 Tip Comb for DW Magnets	Cat. no. 97002534
KingFisher™ Duo 12-Tip Comb, for Microtiter 96 Deepwell plate	Cat. no. 97003500
Other consumables	
For KingFisher™ Duo Prime Magnetic Particle Processor only, KingFisher™ Duo Elution Strip	Cat. no. 97003520
MicroAmp™ Clear Adhesive Film	Cat. no. 4306311
Nonstick, RNase-free Microfuge Tubes (1.5 mL)	Cat. no. AM12450
Nonstick, RNase-free Microfuge Tubes (2.0 mL)	Cat. no. AM12475
5–mL Culture tubes	MLS
Conical tubes (15 mL)	Cat. no. AM12500
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS
1-mL serological syringes	MLS
Reagents	
Isopropanol, 100% (molecular grade or higher)	MLS
Ethanol, 200 proof (absolute)	MLS

[1] See "If needed, download the KingFisher™ Flex or Duo program" on page 10

[2] Not available for sale.

[3] KingFisher™ Duo Combi Pack (Cat. no. 97003530) includes plates and combs for the KingFisher™ Duo Prime Magnetic Particle Processor.

If needed, download the KingFisher™ Flex or Duo program

The program required for this protocol is not pre-installed on the KingFisher™ Flex Magnetic Particle Processor 96DW or on the KingFisher™ Duo Prime Magnetic Particle Processor.

1. On the MagMAX™ FFPE Total Nucleic Acid Isolation Kit product web page, scroll down to the **Product Literature** section.
2. Right-click on the appropriate program:
   - **4463365_RNA** for RNA isolation
   - **4463578_gDNA** for DNA isolation
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.

2 Isolate RNA

Important 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).
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. The same Plate Cover can be used throughout the procedure, unless it becomes contaminated.
- If you use a titer plate shaker other than the Thermo Scientific™ Compact Digital Microplate Shaker, verify that:
  - The plate fits securely on your titer plate shaker.
  - The recommended speeds are compatible with your titer plate shaker. Ideal speeds should allow for thorough mixing without splashing.

- If you use a magnetic stand that collects the Nucleic Acid Binding Beads into a ring around the well, make sure to dry the beads for no longer than 2 minutes. Beads collected into a ring dries faster than beads collected in a pellet.
- Volumes for reagent mixes are given per well. 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 first use: prepare Wash Solutions

- Add 24 mL of 100% isopropanol to Wash Solution 1 Concentrate, then mix well.
- Add 48 mL of 100% ethanol to Wash Solution 2 Concentrate, then mix well.

Store at room temperature.

Isolate RNA using the MagMAX™ Express-96 or KingFisher™ Flex instruments

Before each use: prepare DNase Solution

Prepare the DNase Solution as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
DNase Buffer	196 µL
DNase	4 µL
Total DNase Solution	200 µL

Prepare the samples and add the Nucleic Acid Binding Beads

1. Cut one or two 10-µM FFPE sections, then transfer to a well of a MagMAX™ Express-96 Deep Well Plate for each sample.

   IMPORTANT! Do not use more than two 10-µm sections or a total of 20 µm per sample, as this may result in low yield.

2. Add 150 µL of Digestion Buffer to each sample well and immerse the sections in the buffer.

   To immerse the sections, push them down with the plunger of a 1-mL serological syringe.

3. Add 4 µL of Protease and 30 µL of RNA Digestion Additive to each sample well.

4. Cover the plate and incubate at 60°C for 45 minutes, then at 80°C for 30 minutes.

   IMPORTANT! Ensure that the samples are heated properly by checking the temperature in a well containing water. You may need to set the temperature higher to reach 60°C or 80°C in the wells.

   While the samples are incubating, follow "Set up the processing plates" on page 13.

5. During the incubation, prepare the Binding Solution according to the following table and mix well.

Reagents	Volume per well
Binding Buffer	200 µL
Isopropanol	420 µL
Total Binding Solution	620 µL

6. After the incubation at 80°C is complete, add 620 µL of Binding Solution to each sample well.

7. *(Optional)* Spin the plate for 5 seconds.

8. Let the plate cool for 5 minutes at room temperature.

   Note: If excess paraffin is present in the well, transfer the sample mix to a new plate.

9. Add 20 µL of Nucleic Acid Binding Beads to each sample well, then proceed to "Wash, bind, and elute the RNA" on page 13.

Set up the processing plates

While the samples are incubating, set up the Wash, DNase, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Table 2 Plate setup (MagMAX™ Express-96 or KingFisher™ Flex instruments)

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	400 µL
Wash Plate 2	3	Standard	Wash Solution 2	150 µL
DNase Plate[2]	4	Deep Well	DNase Solution	200 µL
Wash Plate 3	5	Standard	Wash Solution 2	150 µL
Wash Plate 4	6	Standard	Wash Solution 2	150 µL
Elution Plate	7	Standard	Elution Buffer	70 µL
Tip Comb	8	Deep Well or Standard	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 150 µL of Binding Buffer and 350 µL of isopropanol to the DNase Plate after the DNase treatment step.

Wash, bind, and elute the RNA

1. Ensure that the instrument is set up for processing with the deep well magnetic head and select the program on the instrument.
   - **4463365 DW FFPE RNA** on the MagMAX™ Express-96 Deep Well Magnetic Particle Processor
   - **4463365_RNA** on the KingFisher™ Flex Magnetic Particle Processor 96DW

2. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 2).

3. Load the sample plate (containing digested tissue, isopropanol, and Binding Beads Mix) at position 1 when prompted by the instrument.

4. Prepare the Rebinding Solution according to the following table.

Reagents	Volume per well
Binding Buffer	150 µL
Isopropanol	350 µL
Total Rebinding Solution	500 µL

5. When prompted by the instrument (30–35 minutes after the initial start):
   a. Remove the DNase Plate from the instrument.
   b. Add 500 µL of Rebinding Solution to each sample well.
      Add Rebinding Solution immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.
   c. Load the DNase Plate back onto the instrument, and press **Start**.

6. At the end of the run (approximately 60 minutes after the initial start), remove the Elution Plate from the instrument and seal immediately with a new MicroAmp™ Clear Adhesive Film.
   - If excess bead residue is seen in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the RNA.
   - *(Optional)* Eluates can be transferred to a storage plate after collection.

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. Alternatively, store the covered Elution Plate:

- At 4°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Isolate RNA using the KingFisher™ Duo Prime instrument

Before each use: prepare DNase Solution

Prepare the DNase Solution as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
DNase Buffer	196 µL
DNase	4 µL
Total DNase Solution	200 µL

Prepare the samples and add the Nucleic Acid Binding Beads

1. Cut one or two 10-µM FFPE sections, then transfer to a well of row H of a MagMAX™ Express-96 Deep Well Plate for each sample.

   IMPORTANT! Do not use more than two 10-µm sections or a total of 20 µm per sample, as this may result in low yield.

2. Add 150 µL of Digestion Buffer to each sample well and immerse the sections in the buffer.

   To immerse the sections, push them down with the plunger of a 1-mL serological syringe.

3. Add 4 µL of Protease and 30 µL of RNA Digestion Additive to each sample well.

4. Cover the plate and incubate at 60°C for 45 minutes, then at 80°C for 30 minutes.

   IMPORTANT! Ensure that the samples are heated properly by checking the temperature in a well containing water. You may need to set the temperature higher to reach 60°C or 80°C in the wells.

5. During the incubation, prepare the Binding Solution according to the following table and mix well.

Reagents	Volume per well
Binding Buffer	200 µL
Isopropanol	420 µL
Total Binding Solution	620 µL

6. After the incubation at 80°C is complete, add 620 µL of Binding Solution to each sample well.

7. *(Optional)* Spin the plate for 5 seconds.

8. Let the plate cool for 5 minutes at room temperature.

   Note: If excess paraffin is present in the well, transfer the sample mix to a new plate.

9. Add 20 µL of Nucleic Acid Binding Beads to each sample well.

Set up the processing plate and the Elution Strip

1. Add processing reagents as indicated in the following table.

Table 3 Plate setup (KingFisher™ Duo Prime instrument)

Row ID	Plate row[1]	Reagent	Volume per well
DNase[2]	A	DNase Solution	200 µL
Tip Comb	B	Place a KingFisher™ Duo 12-Tip Comb in Row B.	
Wash 4	C	Wash Solution 2	150 µL
Wash 3	D	Wash Solution 2	150 µL
Wash 2	E	Wash Solution 2	150 µL
Wash 1	F	Wash Solution 1	400 µL
	G	Empty	

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

[2] The instrument prompts the user to add 150 µL of Binding Buffer and 350 µL of isopropanol to the DNase Plate after the DNase treatment step.

2. Add 70 µL of Elution Buffer to the wells of an Elution Strip.

Wash, bind, and elute the RNA

1. Ensure that the instrument is set up for processing with the deep well 96–well plates and select the program **4463365_RNA** on the instrument.
2. Start the run and load the prepared processing plate and Elution Strip when prompted by the instrument (see "Set up the processing plate and the Elution Strip" on page 16).
3. Prepare the Rebinding Solution according to the following table.

Reagents	Volume per well
Rebinding Buffer	150 µL
Isopropanol	350 µL
Total Rebinding Solution	500 µL

4. When prompted by the instrument (approximately 30–35 minutes after initial start):
   a. Remove the plate from the instrument.
   b. Add 500 µL of Rebinding Solution to each sample well in Row A.
      Add Binding Solution immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.
   c. Load the plate back onto the instrument, and press **Start**.
5. At the end of the run (approximately 60 minutes after initial start), remove the Elution Strip from the instrument and transfer the eluted RNA to an Elution Plate.

6. 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. Alternatively, store the covered Elution Plate:

- At 4°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Isolate RNA manually

Before each use: prepare DNase Solution

- Pre-heat a sufficient volume of Elution Buffer to 50°C.
- Prepare the DNase Solution as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
DNase Buffer	96 µL
DNase	4 µL
Total DNase Solution	100 µL

Prepare the samples

1. Cut one or two 10-µM FFPE sections, then transfer to a well of the Processing Plate for each sample.

IMPORTANT! Do not use more than two 10-µm sections or a total of 20 µm per sample, as this may result in low yield.

2. Add 150 µL of Digestion Buffer to each sample well and immerse the sections in the buffer.

   To immerse the sections, push them down with the plunger of a 1-mL serological syringe.

3. Add 4 µL of Protease and 30 µL of RNA Digestion Additive to each sample well.

4. Cover the plate and incubate at 60°C for 45 minutes, then at 80°C for 30 minutes.

IMPORTANT! Ensure that the samples are heated properly by checking the temperature in a well containing water. You may need to set the temperature higher to reach 60°C or 80°C in the wells.

5. During the incubation, prepare the Binding Solution according to the following table and mix well.

Reagents	Volume per well
Binding Buffer	200 µL
Isopropanol	420 µL
Total Binding Solution	620 µL

6. After the incubation at 80°C is complete, add 620 µL of Binding Solution to each sample well.
7. *(Optional)* Spin the plate for 5 seconds.
8. Let the plate cool for 5 minutes at room temperature.

   Note: If excess paraffin is present in the well, transfer the sample mix to a new plate.

Bind RNA to the Nucleic Acid Binding Beads

1. Add 20 µL of Nucleic Acid Binding Beads to each sample well, then shake for 3 minutes at speed 3–4.
2. Remove the Processing Plate from the shaker and carefully pipet the samples up and down 10–12 times.
3. Place the Processing Plate on the Magnetic Stand-96 for 3–5 minutes or until the solution is clear.
4. Carefully aspirate and discard the supernatant without disturbing the Nucleic Acid Binding Beads.

Wash the Nucleic Acid Binding Beads

1. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 1 to each sample well.
2. Cover and shake the Processing Plate for 1 minute at speed 8.
3. Place the Processing Plate on the Magnetic Stand-96 for 1–2 minutes or until the solution is clear.
4. Carefully aspirate and discard the supernatant without disturbing the Nucleic Acid Binding Beads.
5. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 2 to each sample well.
6. Cover and shake the Processing Plate for 1 minute at speed 8.
7. Place the Processing Plate on the Magnetic Stand-96 for 1–2 minutes or until the solution is clear.

8. Carefully aspirate and discard the supernatant without disturbing the Nucleic Acid Binding Beads.
9. Shake the uncovered Processing Plate for 2 minutes at speed 9 to dry the Nucleic Acid Binding Beads.

Treat with DNase

1. Add 100 µL of DNase Solution to each sample well, then shake for 2 minutes at speed 8.
2. Cover the Processing Plate and incubate for 10–15 minutes at 37°C.
3. Shake the Processing Plate for 5–10 minutes at speed 8.
4. During the agitation, prepare the Rebinding Solution as indicated in the following table.

Reagents	Volume per well
Rebinding Buffer	75 µL
Isopropanol	175 µL
Total Rebinding Solution	250 µL

5. Add 250 µL of Rebinding Solution to each sample well, then shake for 3 minutes at speed 6–7.
6. Mix the samples by pipetting up and down 10–12 times.
7. Place the Processing Plate on the Magnetic Stand-96 for 3–4 minutes or until the solution is clear.
8. Carefully aspirate and discard the supernatant without disturbing the Nucleic Acid Binding Beads.

Wash and elute the RNA

1. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 2 to each sample well.
2. Cover and shake the Processing Plate for 1 minute at speed 8.
3. Place the Processing Plate on the Magnetic Stand-96 for 1–2 minutes or until the solution is clear.
4. Carefully aspirate and discard the supernatant without disturbing the Nucleic Acid Binding Beads.
5. Repeat step 1–step 4 once.
6. Shake the uncovered Processing Plate for 2 minutes at speed 9 to dry the Nucleic Acid Binding Beads.
7. Add 70 µL of pre-heated Elution Buffer, then cover and shake the Processing Plate for 1 minute at speed 9.

   Note: If the Nucleic Acid Binding Beads are not in suspension after agitation, gently pipet up and down 5–7 times.

8. Incubate the Processing Plate for 4 minutes at 37°C.
9. Shake the Processing Plate for 4 minutes at speed 9.
10. Place the Processing Plate on the Magnetic Stand-96 for 2–3 minutes or until the solution is clear.
11. Transfer the supernatant to a new Elution Plate and seal immediately with a new MicroAmp™ Clear Adhesive Film.

The purified RNA is ready for immediate use. Alternatively, store the covered Elution Plate:

- At 4°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

3 Isolate DNA

Important 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.
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. The same Plate Cover can be used throughout the procedure, unless it becomes contaminated.
- If you use a titer plate shaker other than the Thermo Scientific™ Compact Digital Microplate Shaker, verify that:
  - The plate fits securely on your titer plate shaker.
  - The recommended speeds are compatible with your titer plate shaker. Ideal speeds should allow for thorough mixing without splashing.
- If you use a magnetic stand that collects the Nucleic Acid Binding Beads into a ring around the well, make sure to dry the beads for no longer than 2 minutes. Beads collected into a ring dries faster than beads collected in a pellet.
- Volumes for reagent mixes are given per well. 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 first use: prepare Wash Solutions

- Add 24 mL of 100% isopropanol to Wash Solution 1 Concentrate, then mix well.
- Add 48 mL of 100% ethanol to Wash Solution 2 Concentrate, then mix well.

Store at room temperature.

Isolate DNA using the MagMAX™ Express-96 or KingFisher™ Flex instruments

Before each use: prepare RNase Solution

Prepare the RNase Solution as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
Nuclease-Free Water	199 µL
RNase A	1 µL
Total RNase Solution	200 µL

Prepare the samples and add the Nucleic Acid Binding Beads

1. Cut one or two 10-µM FFPE sections, then transfer to a well of a MagMAX™ Express-96 Deep Well Plate for each sample.

   IMPORTANT! Do not use more than two 10-µm sections or a total of 20 µm per sample, as this may result in low yield.

2. Add 150 µL of Digestion Buffer to each sample well and immerse the sections in the buffer.

   To immerse the sections, push them down with the plunger of a 1-mL serological syringe.

3. Add 4 µL of Protease and 30 µL of DNA Digestion Additive to each sample well.

4. Cover the plate and incubate at 60°C for 60 minutes, then at 80°C for 30 minutes.

   IMPORTANT! Ensure that the samples are heated properly by checking the temperature in a well containing water. You may need to set the temperature higher to reach 60°C or 80°C in the wells.

   While the samples are incubating, follow "Set up the processing plates" on page 23.

5. During the incubation, prepare the Binding Solution according to the following table and mix well.

Reagents	Volume per well
Binding Buffer	200 µL
Isopropanol	420 µL
Total Binding Solution	620 µL

6. After the incubation at 80°C is complete, add 620 µL of Binding Solution to each sample well.

7. *(Optional)* Spin the plate for 5 seconds.

8. Let the plate cool for 5 minutes at room temperature.

   Note: If excess paraffin is present in the well, transfer the sample mix to a new plate.

9. Add 20 µL of Nucleic Acid Binding Beads to each sample well, then proceed to "Wash, bind, and elute the DNA" on page 23.

Set up the processing plates

While the samples are incubating, set up the Wash, RNase, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Table 4 Plate setup (MagMAX™ Express-96 or KingFisher™ Flex instruments)

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	400 µL
Wash Plate 2	3	Standard	Wash Solution 2	150 µL
RNase Plate[2]	4	Deep Well	RNase Solution	200 µL
Wash Plate 3	5	Standard	Wash Solution 2	150 µL
Wash Plate 4	6	Standard	Wash Solution 2	150 µL
Elution Plate	7	Standard	Elution Buffer	70 µL
Tip Comb	8	Deep Well or standard	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 200 µL of Binding Buffer and 400 µL of isopropanol to the RNase Plate after the RNase treatment step.

Wash, bind, and elute the DNA

1. Ensure that the instrument is set up for processing with the deep well magnetic head and select the program on the instrument.
   - **4463365 DW FFPE DNA** on the MagMAX™ Express-96 Deep Well Magnetic Particle Processor
   - **4463578_gDNA** on the KingFisher™ Flex Magnetic Particle Processor 96DW
2. Start the run and load the prepared processing plates in their positions when prompted by the instrument (see Table 4).
3. Load the sample plate (containing digested tissue, isopropanol, and Binding Beads Mix) at position 1 when prompted by the instrument.
4. Prepare the Rebinding Solution according to the following table.

Reagents	Volume per well
Rebinding Buffer	200 µL
Isopropanol	400 µL
Total Rebinding Solution	600 µL

5. When prompted by the instrument (30–35 minutes after the initial start):
   a. Remove the RNase Plate from the instrument.

b. Add 600 µL of Rebinding Solution to each sample well.

   Add Rebinding Solution immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.

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

6. At the end of the run (approximately 60 minutes after the initial start), remove the Elution Plate from the instrument and seal immediately with a new MicroAmp™ Clear Adhesive Film.
   - If excess bead residue is seen in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the DNA.
   - *(Optional)* Eluates can be transferred to a storage plate after collection.

   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. Alternatively, store the covered Elution Plate:

- At 4°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Isolate DNA using the KingFisher™ Duo Prime instrument

Before each use: prepare RNase Solution

Prepare the RNase Solution as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
Nuclease-Free Water	199 µL
RNase A	1 µL
Total RNase Solution	200 µL

Prepare the samples and add the Nucleic Acid Binding Beads

1. Cut one or two 10-µM FFPE sections, then transfer to a well of row H of a MagMAX™ Express-96 Deep Well Plate for each sample.

   IMPORTANT! Do not use more than two 10-µm sections or a total of 20 µm per sample, as this may result in low yield.

2. Add 150 µL of Digestion Buffer to each sample well and immerse the sections in the buffer.

   To immerse the sections, push them down with the plunger of a 1-mL serological syringe.

3. Add 4 µL of Protease and 30 µL of DNA Digestion Additive to each sample well.

4. Cover the plate and incubate at 60°C for 60 minutes, then at 80°C for 30 minutes.

   IMPORTANT! Ensure that the samples are heated properly by checking the temperature in a well containing water. You may need to set the temperature higher to reach 60°C or 80°C in the wells.

5. During the incubation, prepare the Binding Solution according to the following table and mix well.

Reagents	Volume per well
Binding Buffer	200 µL
Isopropanol	420 µL
Total Binding Solution	620 µL

6. After the incubation at 80°C is complete, add 620 µL of Binding Solution to each sample well.

7. *(Optional)* Spin the plate for 5 seconds.

8. Let the plate cool for 5 minutes at room temperature.

   Note: If excess paraffin is present in the well, transfer the sample mix to a new plate.

9. Add 20 µL of Nucleic Acid Binding Beads to each sample well.

Set up the processing plate and the Elution Strip

1. Add processing reagents as indicated in the following table.

   Table 5 Plate setup (KingFisher™ Duo Prime instrument)

Row ID	Plate row[1]	Reagent	Volume per well
RNase[2]	A	RNase Solution	200 µL
Tip Comb	B	Place a KingFisher™ Duo 12-Tip Comb in Row B.	
Wash 4	C	Wash Solution 2	150 µL
Wash 3	D	Wash Solution 2	150 µL
Wash 2	E	Wash Solution 2	150 µL
Wash 1	F	Wash Solution 1	400 µL
	G	Empty	

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

   [2] The instrument prompts the user to add 200 µL of Binding Buffer and 400 µL of isopropanol to the RNase Row after the RNase treatment step.

2. Add 70 µL of Elution Buffer to the wells of an Elution Strip.

Wash, bind, and elute the DNA

1. Ensure that the instrument is set up for processing with the deep well 96–well plates and select the program **4463578_gDNA** on the instrument.
2. Start the run and load the prepared processing plate and Elution Strip when prompted by the instrument (see "Set up the processing plate and the Elution Strip" on page 25).
3. Prepare the Rebinding Solution according to the following table.

Reagents	Volume per well
Rebinding Buffer	200 µL
Isopropanol	400 µL
Total Rebinding Solution	600 µL

4. When prompted by the instrument (approximately 30–35 minutes after initial start):
   a. Remove the plate from the instrument.
   b. Add 600 µL of Rebinding Solution to each sample well in Row A.
      Add Rebinding Solution immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.
   c. Load the plate back onto the instrument, and press **Start**.
5. At the end of the run (approximately 60 minutes after initial start), remove the Elution Strip from the instrument and transfer the eluted DNA to an Elution Plate.
6. 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. Alternatively, store the covered Elution Plate:

- At 4°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Isolate DNA manually

Before each use: prepare RNase Solution

- Pre-heat a sufficient volume of Elution Buffer to 80°C.
- Prepare the RNase Solution as indicated in the following table, mix, and store on ice until use.

Component	Volume per well
Nuclease-Free Water	99 µL
RNase A	1 µL
Total RNase Solution	100 µL

Prepare the samples

1. Cut one or two 10-µM FFPE sections, then transfer to a well of the Processing Plate for each sample.

   IMPORTANT! Do not use more than two 10-µm sections or a total of 20 µm per sample, as this may result in low yield.

2. Add 150 µL of Digestion Buffer to each sample well and immerse the sections in the buffer.

   To immerse the sections, push them down with the plunger of a 1-mL serological syringe.

3. Add 4 µL of Protease and 30 µL of DNA Digestion Additive to each sample well.

4. Cover the plate and incubate at 60°C for 60 minutes, then at 80°C for 30 minutes.

   IMPORTANT! Ensure that the samples are heated properly by checking the temperature in a well containing water. You may need to set the temperature higher to reach 60°C or 80°C in the wells.

5. During the incubation, prepare the Binding Solution according to the following table and mix well.

Reagents	Volume per well
Binding Buffer	200 µL
Isopropanol	420 µL
Total Binding Solution	620 µL

6. After the incubation at 80°C is complete, add 620 µL of Binding Solution to each sample well.

7. *(Optional)* Spin the plate for 5 seconds.

8. Let the plate cool for 5 minutes at room temperature.

   Note: If excess paraffin is present in the well, transfer the sample mix to a new plate.

Bind DNA to the Nucleic Acid Binding Beads

1. Add 20 µL of Nucleic Acid Binding Beads to each sample well, then shake for 3 minutes at speed 3–4.
2. Remove the Processing Plate from the shaker and carefully pipet the samples up and down 10–12 times.
3. Place the Processing Plate on the Magnetic Stand-96 for 3–5 minutes or until the solution is clear.
4. Carefully aspirate and discard the supernatant without disturbing the Nucleic Acid Binding Beads.

Wash the Nucleic Acid Binding Beads

1. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 1 to each sample well.
2. Cover and shake the Processing Plate for 1 minute at speed 8.
3. Place the Processing Plate on the Magnetic Stand-96 for 1–2 minutes or until the solution is clear.
4. Carefully aspirate and discard the supernatant without disturbing the Nucleic Acid Binding Beads.
5. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 2 to each sample well.
6. Cover and shake the Processing Plate for 1 minute at speed 8.
7. Place the Processing Plate on the Magnetic Stand-96 for 1–2 minutes or until the solution is clear.
8. Carefully aspirate and discard the supernatant without disturbing the Nucleic Acid Binding Beads.
9. Shake the uncovered Processing Plate for 2 minutes at speed 9 to dry the Nucleic Acid Binding Beads.

Treat with RNase A

1. Add 100 µL of RNase Solution to each sample well, then shake for 2 minutes at speed 8.
2. Cover the Processing Plate and incubate for 20 minutes at 37°C.
3. Shake the Processing Plate for 5–10 minutes at speed 8.
4. During the agitation, prepare the Rebinding Solution as indicated in the following table.

Reagents	Volume per well
Rebinding Buffer	100 µL
Isopropanol	200 µL
Total Rebinding Solution	300 µL

5. Add 300 µL of Rebinding Solution to each sample well, then shake for 3 minutes at speed 6–7.
6. Mix the samples by pipetting up and down 10–12 times.
7. Place the Processing Plate on the Magnetic Stand-96 for 3–4 minutes or until the solution is clear.
8. Carefully aspirate and discard the supernatant without disturbing the Nucleic Acid Binding Beads.

Wash and elute the DNA

1. Remove the Processing Plate from the Magnetic Stand-96 and add 150 µL of Wash Solution 2 to each sample well.
2. Cover and shake the Processing Plate for 1 minute at speed 8.
3. Place the Processing Plate on the Magnetic Stand-96 for 1–2 minutes or until the solution is clear.
4. Carefully aspirate and discard the supernatant without disturbing the Nucleic Acid Binding Beads.
5. Repeat step 1–step 4.
6. Shake the uncovered Processing Plate for 2 minutes at speed 9 to dry the Nucleic Acid Binding Beads.
7. Add 70 µL of pre-heated Elution Buffer, then cover and shake the Processing Plate for 1 minute at speed 9.

   Note: If the Nucleic Acid Binding Beads are not in suspension after agitation, gently pipet up and down 5–7 times.
8. Incubate the Processing Plate for 4 minutes at 80°C.
9. Shake the Processing Plate for 4 minutes at speed 9.
10. Place the Processing Plate on the Magnetic Stand-96 for 3–8 minutes or until the solution is clear.
11. Transfer the supernatant to a new Elution Plate and seal immediately with a new MicroAmp™ Clear Adhesive Film.

The purified DNA is ready for immediate use. Alternatively, store the covered Elution Plate:

- At 4°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

A

Troubleshooting

Observation	Possible cause	Recommended action
Nucleic acid appears fragmented	Overheating during Protease digestion	Make sure that the temperature of the incubation device does not fluctuate significantly.
	RNA is degraded after purification	See "Important procedural guidelines" on page 11 for recommendations for handling RNA.
Low yield	Tissues can vary enormously in their nucleic acid content and in extraction efficiency. Very fibrous tissues, such as muscle, will tend to form a more tightly-crosslinked web upon fixation, so relative recovery will be lower.	After adding the Binding Solution, transfer the samples to a new plate before proceeding with isolation.
		Increase sample mixing by pipetting the samples up and down before proceeding with automated protocols, or increase the number of up-and-down pipettings for the manual protocol.
		For DNA, try extending the incubation up to 2 hours for both the 60°C and 80°C temperatures.
	Tissue was not digested	Ensure that the entire section is fully immersed in the Digestion Buffer. **Note:** The rubber end of a plunger of a 1-mL serological syringe is best suited for immersing the entire sample in the Digestion Buffer.
		Ensure that the heat source is heating the sample at the correct temperature by checking the temperature of the liquid in the plate.
	Too much tissue was added to the digestion reaction	Do not use more than 2 10-µm sections or a total of 20 µm per sample.
	Too much digestion additive in the reaction	Do not use more than 30 µL of digestion additive.
	The Binding Beads Mix was stored incorrectly	Store the Binding Beads Mix at room temperature for immediate use, or at 2–8°C for long-term storage. **IMPORTANT! Do not freeze the Binding Beads Mix.**

Observation	Possible cause	Recommended action
Low yield *(continued)*	The amount of Nucleic Acid Binding Beads added was not sufficient	Vortex the tube containing the Nucleic Acid Binding Beads thoroughly, immediately before use.
	The bead pellet was lost during the binding of washing steps	When removing the supernatant, angle the pipette tip away from the bead pellet.
		Place the plate on the magnetic stand for the recommended times.
	The Nucleic Acid Binding Beads were added before the isopropanol	Add the binding beads after adding the isopropanol and Binding Buffer.
	Binding was incomplete	Ensure that the samples are mixed well before collecting the beads. Pipetting up and down after the initial plate shaking ensures complete mixing.
	The volume of isopropanol was incorrect	Use the volumes called for in the protocol.
	Nucleic acid is still bound to the beads, or the beads remain clumped after elution	Increase the volume of Elution Buffer.
		Increase the speed of the shaker used during the elution step. Pipet up and down to break apart the bead pellets.
	The elution conditions were incorrect	Use only the MagMAX™ Elution Buffer.
		For the manual protocol, always preheat the Elution Buffer to the recommended temperature.
	Samples did not go through the rebinding step	Verify that the samples were mixed with the correct volumes of Binding Solution after the nuclease treatment.
Reduced downstream functionality	The ability of recovered nucleic acid to function in downstream applications can be reduced depending on the quality of the sample.	For RNA: • Use random primers or gene-specific primers in the RT reaction. • Use smaller amplicons for real-time PCR, or increase the mass input into the reactions.
		For DNA: • Use qPCR primer/probe sets with smaller amplicons. • Increase the incubation time of the 60°C and 80°C incubations up to 2 hours total. This can improve downstream functionality of larger PCR amplicons.

Observation	Possible cause	Recommended action
Contamination	DNA contamination of RNA	Reduce the size of the sample.
		Perform an additional DNase treatment after RNA isolation. We recommend using the TURBO DNA-*free*™ Kit (Cat. no. AM1907).
		Use only the provided DNase and DNase Buffer provided in the kit.
	RNA contamination of DNA	Perform an additional RNase treatment after DNA isolation. We recommend using RNase A (RPA grade; Cat. no. AM2272)).
	Paraffin residue carryover	Trim away excess paraffin from the sample prior to digestion.
		After adding the Binding Solution, transfer samples to a new plate before proceeding with isolation.
	Salt carryover	Verify that the correct type and volume of alcohol is added to wash concentrate.
	Alcohol carryover	For the manual protocol, verify that the alcohol has evaporated before adding the Elution Buffer (sample is not wet).
	Other contaminants	Precipitate with ethanol to further purify the recovered nucleic acid: 1. Add ammonium acetate to a final concentration of 2–2.5 M and mix well. 2. Add 4 volumes of ethanol and mix well. 3. Incubate at –20°C (or lower) for at least 30 minutes. To maximize miRNA recovery, incubate at –20°C for at least 12 hours. 4. Centrifuge at 16,000 × *g* for 20–30 minutes to pellet the nucleic acid. 5. Wash the pellet twice with 80% ethanol. 6. Resuspend the pellet in Nuclease-Free Water or TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

Observation	Possible cause	Recommended action
The nucleic acid yield varies from well to well	Variability of FFPE tissue samples	Process replicate sections for each tissue.
	The Nucleic Acid Binding Beads were not fully resuspended/dispersed	Resuspend the binding beads at temperatures above 20°C.
		For the manual protocol, ensure that the beads are fully dispersed by pipetting up and down a minimum of 10 times.
	Sample evaporation	If the samples are stored for too long and evaporation occurs, immediately add Nuclease-Free Water to the correct volume.
		Cover the plate immediately after transferring the eluate, or removing the plate or strip from the automated platform. **Note:** The elution plates or strips are not meant for long-term storage or archiving. Transfer samples to the appropriate container for sample archiving.

Factors affecting nucleic acid recovery and integrity

Tissue fixation and embedding causes nucleic acid modifications

During the tissue fixation process, formaldehyde reacts primarily with the nitrogen atoms of lysine, arginine, and histidine, resulting in extensive cross-linking of the protein matrix within each cell. Some of these crosslinks extend to the nucleic acid species present, primarily through the exocyclic nitrogens of adenine and cytidine. The subsequent embedding process further accelerates this chemistry by heating the sample, often in the formalin solution prior to the soak in hot paraffin.

The resulting extensive web of protein-protein and protein-nucleic acid crosslinks tends to physically entrap nucleic acid species, even those that are not chemically crosslinked. Protease treatment breaks down this web and frees the nucleic acid. Even after Protease treatment, some of the nucleic acid bases will still be crosslinked to amino acids, peptides, or other biomolecules.

There may also be other, unidentified, formaldehyde-induced modifications. Any sites of modification on the nucleic acid chain provide a block to polymerases using it as a template.

The degree of nucleic acid fragmentation varies

Nucleic acid modifications also contribute to the fragmentation of DNA over time, even while stored in the block of paraffin. For this reason, the average size of DNA obtained from FFPE samples can vary widely. One key factor that contributes to DNA quality appears to be removal of all excess formaldehyde during the original embedding procedure. Another is storage of blocks without cut faces, which prevents damage from atmospheric oxygen, water and other environmental factors such as light and infestation (fungus, insects, etc.).

The extent of modification is apparent from a diminished function as a template for polymerases; quantitative PCR (RT or standard) will often show an increase in the cycle threshold of 5–6 cycles (with equivalent mass inputs) compared to DNA from unfixed tissue.

DNA recovered from FFPE samples tends to be much less fragmented than RNA, but still exhibits a broad size distribution, and has diminished capacity to serve as a template for polymerases.

B

Assessing yield and integrity

Assess RNA yield and integrity

Assess RNA yield

Spectrophotometry

The concentration of an RNA solution can be determined by measuring its absorbance at 260 nm. We recommend using the NanoDrop™ 1000/8000 Spectrophotometer. Measure 2 µL of the RNA sample directly.

Fluorometry

If a fluorometer or a fluorescence microplate reader is available, Qubit™ fluorescence-based assay for RNA quantitation is a convenient and sensitive way to measure RNA concentration.

Assess RNA quality

Spectrophotometry

The A_{260}/A_{280} ratio of the RNA is an indication of its purity. The RNA isolated with this protocol should have an A_{260}/A_{280} ratio of 1.7–2.1.

Agarose gel electrophoresis

You can also assess the quality of your RNA sample by agarose gel electrophoresis.

Note: Typically, RNA recovered from FFPE samples will appear smeared.

Microfluidics analysis

The Agilent™ 2100 Bioanalyzer™, in conjunction with an RNA LabChip® Kit, provides a powerful and sensitive method to assess RNA integrity. To use this system, follow the instructions for RNA analysis provided with the RNA LabChip® Kit.

Most preparations will show a broad peak that ranges in size from 80–350 nucleotides.

Recovery of miRNA

Fragmentation of FFPE RNA seems to have a size endpoint of ~80 nucleotides, and modifications affect only about 1% of the nucleotides. Thus, miRNA molecules, which are 21–23 nucleotides, are basically untouched by the fixation/embedding process. Using sample sections of ≥10 µm thickness will minimize loss of miRNA.

Potential modifications to downstream applications

The purity of the recovered nucleic acid is sufficient for most applications. Nucleic acid fragmentation and some chemical modifications, caused by sample fixation and storage, will remain after the procedure. Therefore, some downstream procedures may need to be modified for best results.

Real-time PCR

The recovered RNA can be used in real-time PCR analysis. Because the RNA extracted from fixed tissues is likely to be degraded, plan to analyze small amplicons. RNA from FFPE samples always requires more PCR cycles to produce the same signal as RNA from frozen tissues from the same source. This is probably due to nucleic acid modifications incurred by the fixation process.

Array analysis

While recovered RNA can be amplified for use in array analysis using, for example, the MessageAmp™ Premier Kit (Cat. no. AM1792), there are several caveats. Since amplification procedures prime from the 3' end of mRNA, the fragmentation and presence of formaldehyde-induced lesions on the RNA recovered with this kit will create a population of targets that are weighted to the extreme 3' ends of the messages. By using appropriate probes on the arrays, or by applying a filter to the data so that only 3' end-most probes are analyzed, biologically relevant information can be obtained. Even with these compensatory steps, older samples (archived for ≥10 years) will often be so highly fragmented that the number of expressed genes present will be drastically reduced.

Assess DNA yield and integrity

Assess DNA yield

DNA yield and quality depends on the tissue and fixing procedure. The concentration of a DNA solution can be determined by measuring its absorbance at 260 nm. We recommend using the NanoDrop™ 1000/8000 Spectrophotometer. Measure 2 µL of the DNA sample directly. Alternatively, determine the DNA concentration by diluting an aliquot of the preparation in TE and reading the absorbance at 260 nm in a traditional spectrophotometer. With a path length of 1 cm, an A_{260} of 1 is equivalent to 50 µg of double-stranded DNA/mL. Calculate the DNA concentration (µg/mL) as follows:

A_{260} × dilution factor × 50 µg/mL = µg DNA/mL

Assess DNA quality

Spectrophotometry

The A_{260}/A_{280} ratio of the DNA is an indication of its purity. The DNA isolated with this protocol has an A_{260}/A_{280} ratio of 1.6–2.1.

Quantitative real-time PCR vs. a DNA standard

The ability of recovered DNA to function as a template for PCR can be measured using the TaqMan™ DNA Template Reagents Kit (Cat. no. 401970) and the TaqMan™ RNase P Detection Reagents Kit (Cat. no. 4316831). For further information, refer to *DNA Genotyping from Human FFPE Samples-Reliable and Reproducible* (Pub. no. 137AP04-01).

Agarose gel electrophoresis

The DNA obtained from this procedure will always be a population of sheared fragments. You can assess the average size of this population using standard electrophoretic methods. This will require the use of ~ 0.25–1 µg of DNA, which may be a sizeable fraction of the yield for some samples.

Microfluidics analysis

A bioanalyzer, such as the Agilent™ 2100 Bioanalyzer™, requires a much smaller amount of the recovered DNA than does agarose gel electrophoresis. The recovered DNA typically appears as a broad *smear*, covering a size range up to thousands of base pairs, with the modal size varying from less than 100–~3000 nucleotides, depending on the age and condition of the original sample.

Potential modifications to downstream applications

DNA recovered with the kit can be used in PCR analysis and other downstream applications. Nucleic acid fragmentation and some chemical modifications, caused by sample fixation and storage, will remain after the procedure. Consequently, some downstream procedures may need to be modified for best results. Modifications from the fixation and embedding process occur on average every 100–200 nucleotides; therefore, we recommend analysis of amplicons around 100 nucleotides or smaller.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Related documentation

Document	Publication number
MagMAX™ FFPE DNA Isolation Kit User Guide	4461286
MagMAX™ FFPE RNA Isolation Quick Reference	100033279
MagMAX™ FFPE DNA Isolation Quick Reference	100033280

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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