MagMAX™ FFPE DNA/RNA Ultra Kit

Automated or manual isolation of DNA from FFPE samples

Catalog Number A31881

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

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 Autopliers 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 isolation of DNA 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 sequential DNA and RNA 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 (RNA isolation only)* (Pub. No. MAN0015906), respectively.

Contents and storage

Reagents provided in the kit are sufficient for 96 DNA isolations from sections up to 40 $\mu m.$

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	15 0 10 30 0
Nucleic Acid Binding Beads ^[2]	1.95 mL	2°C to 8°C
DNA Wash Buffer ^[1]	38.5 mL	
Wash Solution 2 Concentrate	210 mL ^[3]	15°C to 30°C
Elution Solution	5 mL	15 0 10 30 0
RNA Wash Buffer Concentrate ^[4]	115 mL	
DNase [4]	1.95 mL	-25°C to -15°C
DNase buffer [4]	960 µL	-25 C 10 -15 C

^[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.

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 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



^[2] Shipped at room temperature.

^[3] Final volume; see "Before first use of the kit" on page 2.

^[4] Not used in this workflow

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 pro	ocessor used:		
KingFisher™ 96 tip comb for DW magnets	97002534		
KingFisher™ Duo 12-tip Comb, for 96 deep-well plate 9700350			
KingFisher™ Apex 96 Combi tip comb 97002			
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.
- 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	A31881_DUO_lg_vol _DNA
KingFisher™ Flex	A31881_FLEX_std_DNA	A31881_FLEX_lg_vol _DNA

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

- 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.
- 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_lg_vol _DNA_v1

- Select the instrument where you want to transfer the protocol, then click Transfer.
- 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.
- 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

 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

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	1.1.	Cut sections from FFPE tissue blocks using a microtome.
	blocks	1.2.	Collect each section in a 1.5-mL microcentrifuge tube.
2	Remove paraffin from the	2.1.	Preheat a heating block (with lid) or incubator at 50°C.
_	sections	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 μ L 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 resul 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.

 $\textbf{5.3.} \ \ \text{Incubate at } 55^{\circ}\text{C for 1 hour or longer, then centrifuge briefly to collect any condensation droplets}.$

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 plate" on page 5.
- For isolation using KingFisher[™] Flex Magnetic Particle Processor 96DW, proceed to "Set up the DNA processing plates" on page 6.
- For isolation using KingFisher™ Apex Purification System with 96 DW Head, proceed to "Set up the DNA processing plates" on page 7.

Allow samples to cool down before proceeding to next step.

- Remove paraffin from the sections
- Remove paraffin from the 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 plate" on page 5.
- For isolation using KingFisher[™] Flex Magnetic Particle Processor 96DW, proceed to "Set up the DNA processing plates" on page 6.
- For isolation using KingFisher[™] Apex Purification System with 96 DW Head, proceed to "Set up the DNA processing plates" on page 7.

Allow samples to cool down before proceeding to next step.

Isolate DNA

- To isolate DNA manually, proceed to "Isolate DNA manually" on page 4.
- To isolate DNA using the KingFisher[™] Duo Prime Magnetic Particle Processor, proceed to "Isolate DNA using KingFisher[™] Duo Prime Magnetic Particle Processor" on page 5.
- To isolate DNA using the KingFisher[™] Flex Magnetic Particle Processor 96DW, proceed to "Isolate DNA using KingFisher[™] Flex Magnetic Particle Processor 96DW" on page 6.
- To isolate DNA using the KingFisher[™] Apex Purification System with 96 DW Head, proceed to "Isolate DNA using KingFisher[™] Apex Purification System with 96 DW Head" on page 7.

Isolate DNA manually

Use microcentrifuge tubes to perform manual DNA isolations.

Bind DNA to the beads

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.

1.2. 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.

Bind DNA to the beads (continued)

- 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 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.

Elute the DNA

- 3.1. Add 50 μ L 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.

Isolate DNA using KingFisher™ Duo Prime Magnetic Particle Processor

Set up the processing plate

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

Table 7 DNA plate setup

Row ID	Plate row ^[1]	Reagent	Volume per well	
HOW ID	riale low-	neagent	Sections ≤40 µm	Sections >40 µm
Sample	А	DNA Binding Buffer (see Table 6)	155 μL	270 μL
DNA Wash Buffer 1	В	DNA Wash Buffer	200 μL	400 μL
DNA Wash Buffer 2	С	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	Н	Elution Solution	50 μL	

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

DNA

- Bind, wash, and elute the 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 for sections ≤40 μm.
 - A31881_DUO_lg_vol_DNA 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 7).

Bind, wash, and elute the DNA (continued)

2.4. At the end of the run, remove the Elution Plate from the instrument and transfer the eluted DNA (Row H) 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 DNA is ready for immediate use. Store at -20°C or -80°C for long-term storage.

Isolate DNA 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 8 DNA plates setup

				Volume per well	
Plate ID	Plate position ^[1]	Plate type	Reagent	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.

- 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_Ig_vol_DNA 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 8).
- 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.

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 9 DNA plates setup

				Volume	per well
Plate ID	Plate position ^[1]	Plate type	Reagent	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.

- 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_Ig_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 8).
- 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.

Limited product warranty

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Revision Date Description		Description	
	C.0	19 April 2021	Addition of protocol for KingFisher™ Apex Purification System with 96DW Head.
	B.0	26 February 2018	Addition of publication titles and numbers for user guides using AutoLys M tubes.
	A.0	12 July 2016	New document.

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