MagMAX™ DNA Multi-Sample Ultra 2.0 Kit

High throughput isolation of DNA from saliva

Catalog Number A36570

Pub. No. MAN0017324 Rev. F

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™ DNA Multi-Sample Ultra 2.0 Kit is developed for scalable, rapid purification of high-quality DNA from a variety of sample matrices. DNA purified with this kit can be used in a broad range of molecular biology downstream applications, such as sequencing, genotyping, and qPCR. This protocol guides through automated isolation of DNA from saliva using the KingFisher™ Flex, KingFisher™ Apex, and the KingFisher™ Duo Prime.

Contents and storage

Reagents provided in the kit are sufficient for 200 reactions using small volume (50–200 µL) inputs.

Table 1 MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (Cat. No. A36570)

Component	Quantity	Storage
Enhancer Solution	4.5 mL	15–30°C
Proteinase K	4.5 mL	
Binding Solution	45 mL	
DNA Binding Beads	4.5 mL	
Wash I Solution	110 mL	
Elution Solution	12 mL	

For 1,000 reaction volume use Cat. No. A36578 (Proteinase K), A36579 (DNA Binding Beads), A36580 (Wash I Solution), A36581 (Lysis/Binding Solution), A36582 (Elution Solution), and A36583 (Enhancer Solution).

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.

Item	Source
Instrument	
Magnetic particle processor (one of the following, depending on quantity/volume of sample to be processed):	
KingFisher™ Duo Prime Magnetic Particle Processor	5400110
For small volume sample[1]: KingFisher™ Flex Magnetic Particle Processor 96DW with 96 deep-well head	5400630
For large volume sample[2]: KingFisher™ Flex Magnetic Particle Processor 24DW with 24 deep-well head	5400640
For small volume sample[1] KingFisher™ Apex with 96 deep-well head	5400930
For large volume sample[2] KingFisher™ Apex with 24 Combi head	5400940

Item	Source
Consumables	
Deep-well plates:	
For small volume sample[1], one of the following: : • KingFisher™ 96 Deep-Well Plates • KingFisher™ 96 Deep-Well Plates, barcoded	 • 95040450 • 95040450B
For large volume sample[2]: KingFisher™ Flex 24 Deep-Well Plates	95040470
96-well standard plates (for use with KingFisher™ Flex and KingFisher™ Apex only; tip comb placement and eluate storage):	
KingFisher™ 96 KF microplate	97002540
Tip comb, compatible with the magnetic particle processor used:	
KingFisher™ Duo Prime 12-tip comb, for use with KingFisher™ 96 Deep-Well Plates	97003500
KingFisher™ Duo Prime 6-tip comb, for use with KingFisher™ Flex 24 Deep-Well Plates	97003510
KingFisher™ 96 tip comb for deep-well magnets (KingFisher™ Flex and KingFisher™ Apex protocols only)	97002534
KingFisher™ Flex 24 deep-well tip comb and plate (KingFisher™ Flex and KingFisher™ Apex protocols only)	97002610
Elution strip (for use with KingFisher™ Duo Prime only; elution step):	
KingFisher™ elution strip for 12 pin magnet	97003520
KingFisher™ elution strip cap for 12 pin magnet	97003540
Equipment	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Reagents	
Ethanol, 96–100% (molecular biology grade)	MLS
Nuclease-free water	AM9932
Materials	
MicroAmp™ Clear Adhesive Film	4306311

[1] Small volume saliva is 100–400 µL.
[2] Large volume saliva is 500 µL–2000 µL.

General guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Precipitates and high viscosity can occur if Enhancer Solution and Binding Solution are stored when room temperature is too cold. If there are precipitates in these solutions, warm them at 37°C and gently mix to dissolve precipitates. Avoid creating bubbles.
- Yellowing of the Binding and Wash I Solution is normal and will not affect buffer performance
- Per-plate volumes for reagent mixes are sufficient for one plate plus overage. To calculate volumes for other sample numbers, refer to the per-well volume and add 10% overage.
- When isolating from a preserved saliva sample (examples include, but are not limited to Oragene™ and GeneFiX™) Enhancer Solution may need to be omitted from the workflow.
- *(Optional)*: To prevent evaporation and contamination, cover the prepared processing plates with paraffin film or MicroAmp™ Clear Adhesive Film until they are loaded into the instrument.

Guidelines for Proteinase K digestion

- Do not pre-mix the Enhancer Solution and Proteinase K.
- Do not change the order of pipetting.

Guidelines for DNA Binding Bead Mix

- Vortex the DNA Binding Beads thoroughly, combine them with the Binding Solution in a nuclease-free tube, then invert the tube until homogeneous. This mixture can be stored for up to 1 day before aliquoting into the plates.
- Ensure that the beads stay fully mixed within the solution during pipetting.
- Avoid creating bubbles during mixing and aliquoting.

Before first use of the kit

Prepare Wash II Solution: Make 80% ethanol from 100% absolute ethanol and Nuclease-Free Water.

IMPORTANT! The Wash I Solution and Lysis/Binding Solution may develop inert white or brown particulates that float in the solution. Visual particulate is not a cause for concern and does not negatively affect performance.

Before each use of the kit

Vortex DNA Binding Beads to fully resuspend the beads before each use.

Perform DNA purification using KingFisher™ Flex (small volume: 100–400 µL)

1 Set up the instrument

1. Ensure that the instrument is set up for processing with the proper magnetic head (96 deep-well) for your application.
2. Ensure that the proper heat block (96 deep-well, not standard) is installed for your application.
3. Ensure that the proper program (**MagMAX_Ultra2_200µL_FLEX** or **MagMAX_Ultra2_400µL_FLEX**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
100–200 µL saliva input				
Wash I Solution Plate	2	Deep Well	Wash I Solution	500 µL
Wash II Solution Plate 1	3	Deep Well	Wash II Solution	500 µL
Wash II Solution Plate 2	4	Deep Well	Wash II Solution	500 µL
Elution Plate	5	Deep Well	Elution Solution	50–100 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate		
200–400 µL saliva input				
Wash I Solution Plate	2	Deep Well	Wash I Solution	1000 µL
Wash II Solution Plate 1	3	Deep Well	Wash II Solution	1000 µL
Wash II Solution Plate 2	4	Deep Well	Wash II Solution	500 µL
Elution Plate	5	Deep Well	Elution Solution	50–100 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate		

Note: The plates will be loaded onto the instrument immediately after the Sample Plate has been prepared.

3 Prepare Sample Plate and digest with Proteinase K

1. Transfer appropriate amount (according to the following table) of Enhancer Solution first, then sample, and lastly Proteinase K, to the appropriate wells of a deep-well plate. This plate will be the Sample Plate.

Enhancer Solution (µL) [1]	Sample Volume (µL) [2]	Proteinase K (µL)
10	100	10
20	200	20
30	300	30
40	400	40

[1] If you are isolating from stabilized saliva, Enhancer Solution may not be needed.
[2] Volumes are referring to total sample input, regardless of the use of preservative.

Note:

- Do not pre-mix the Enhancer Solution and Proteinase K.
- Do not change the order of pipetting.
- Once all components are added, proceed immediately to the instrument processing. There is no need for manual mixing beforehand.

3 *(continued)*

2. Select the program on the instrument according to the following table.

For saliva input volume	Program
100–200 µL	**MagMAX_Ultra2_200µL_FLEX**
200–400 µL	**MagMAX_Ultra2_400µL_FLEX**

3. Start the run, and load the prepared plates into position when prompted by the instrument.

   During this on board Proteinase K sample digestion (~20 minutes) prepare the DNA Binding Bead Mix.

4 Purify the gDNA

1. Prepare DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate
100–200 µL saliva input (96 samples per plate)		
Binding Solution	200 µL	21.12 mL
DNA Binding Beads	20 µL	2.11 mL
Total volume	**220 µL**	**23.23 mL**
200–400 µL saliva input (96 samples per plate)		
Binding Solution	400 µL	42.24 mL
DNA Binding Beads	40 µL	4.22 mL
Total volume	**440 µL**	**46.46 mL**

2. When instructed by the instrument (~20 minutes after the run has started), remove the Sample Plate and add DNA Binding Bead Mix to each sample, according to the following table.

For saliva input volume	Add DNA Binding Bead Mix
100–200 µL	220 µL
200–400 µL	440 µL

   Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.

   IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

3. Immediately place the plate back onto the KingFisher™ Flex and follow the prompts on the instrument to allow the sample processing to proceed.

4. At the end of the run, immediately remove the plates from the instrument and transfer the eluate to the final tube/plate of choice for final storage.

The purified DNA is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Perform DNA purification using KingFisher™ Apex (small volume: 100–400 µL)

1 Set up the instrument

1. Ensure that the instrument is set up for processing with the proper magnetic head (96 deep-well) for your application.

2. Ensure that the proper heat block (96 deep-well, not standard) is installed for your application.

3. Ensure that the proper program (**MagMAX_Ultra2_200µL** or **MagMAX_Ultra2_400µL**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
100–200 µL saliva input				
Wash I Solution Plate	3	Deep Well	Wash I Solution	500 µL
Wash II Solution Plate 1	4	Deep Well	Wash II Solution	500 µL
Wash II Solution Plate 2	5	Deep Well	Wash II Solution	500 µL
Elution Plate	6	Deep Well	Elution Solution	50–100 µL
Tip Comb	1	Place a 96 Deep-well Tip Comb in a Standard Plate		
200–400 µL saliva input				
Wash I Solution Plate	3	Deep Well	Wash I Solution	1000 µL
Wash II Solution Plate 1	4	Deep Well	Wash II Solution	1000 µL
Wash II Solution Plate 2	5	Deep Well	Wash II Solution	500 µL
Elution Plate	6	Deep Well	Elution Solution	50–100 µL
Tip Comb	1	Place a 96 Deep-well Tip Comb in a Standard Plate		

Note: The plates will be loaded onto the instrument immediately after the Sample Plate has been prepared.

3 Prepare Sample Plate and digest with Proteinase K

1. Transfer appropriate amount (according to the following table) of Enhancer Solution first, then sample, and lastly Proteinase K, to the appropriate wells of a deep-well plate. This plate is the Sample Plate.

Enhancer Solution (µL) [1]	Sample Volume (µL) [2]	Proteinase K (µL)
10	100	10
20	200	20
30	300	30
40	400	40

[1] If you are isolating from stabilized saliva, Enhancer Solution may not be needed.
[2] Volumes are referring to total sample input, regardless of the use of preservative.

Note:

- Do not pre-mix the Enhancer Solution and Proteinase K.
- Do not change the order of pipetting.
- Once all components are added, proceed immediately to the instrument processing. There is no need for manual mixing beforehand.

2. Select the program on the instrument according to the following table.

For saliva input volume	Program
100–200 µL	**MagMAX_Ultra2_200µL**
200–400 µL	**MagMAX_Ultra2_400µL**

3. Start the run, and load the prepared plates into position when prompted by the instrument.

   During this on board Proteinase K sample digestion (~20 minutes) prepare the DNA Binding Bead Mix.

4 Purify the gDNA

1. Prepare DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate
100–200 µL saliva input (96 samples per plate)		
Binding Solution	200 µL	21.12 mL
DNA Binding Beads	20 µL	2.11 mL
Total volume	**220 µL**	**23.23 mL**
200–400 µL saliva input (96 samples per plate)		
Binding Solution	400 µL	42.24 mL
DNA Binding Beads	40 µL	4.22 mL
Total volume	**440 µL**	**46.46 mL**

2. When instructed by the instrument (~20 minutes after the run has started), remove the Sample Plate and add DNA Binding Bead Mix to each sample, according to the following table.

For saliva input volume	Add DNA Binding Bead Mix
100–200 µL	220 µL
200–400 µL	440 µL

Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

3. Immediately place the plate back onto the KingFisher™ Apex and follow the prompts on the instrument to allow the sample processing to proceed.

4. At the end of the run, immediately remove the plates from the instrument and transfer the eluate to the final tube/plate of choice for final storage.

The purified DNA is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Perform DNA purification using KingFisher™ Flex (large volume: 500 µL to 2 mL)

1 Set up the instrument

1. Ensure that the instrument is set up for processing with the proper magnetic head (24 deep-well) for your application.

2. Ensure that the proper heat block (24 well) is installed for your application.

3. Ensure that the proper program (**MagMAX_Ultra2_1mL_FLEX** or **MagMAX_Ultra2_2mL_FLEX**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
500 µL – 1 mL saliva input				
Wash I Solution Plate	2	Deep Well	Wash I Solution	2500 µL
Wash II Solution Plate 1	3	Deep Well	Wash II Solution	2500 µL
Wash II Solution Plate 2	4	Deep Well	Wash II Solution	1000 µL
Elution Plate	5	Deep Well	Elution Solution	150–200 µL
Tip Comb	6	KingFisher™ Flex 24 Deep-well Tip Comb and Plate		
1.1 mL – 2 mL saliva input				
Wash I Solution Plate	2	Deep Well	Wash I Solution	5000 µL
Wash II Solution Plate 1	3	Deep Well	Wash II Solution	4000 µL
Wash II Solution Plate 2	4	Deep Well	Wash II Solution	2000 µL
Elution Plate	5	Deep Well	Elution Solution	250–300 µL
Tip Comb	6	KingFisher™ Flex 24 Deep-well Tip Comb and Plate		

Note: The plates will be loaded onto the instrument immediately after the Sample Plate has been prepared.

3 Prepare Sample Plate and digest with Proteinase K

1. Transfer appropriate amount (according to the following table) of Enhancer Solution first, then sample, and lastly Proteinase K, to the appropriate wells of a deep-well plate. This will be the Sample Plate.

Enhancer Solution (µL) [1]	Sample Volume (µL) [2]	Proteinase K (µL)
50	500	50
100	1000	100
200	2000	200

[1] If you are isolating from stabilized saliva, Enhancer Solution may not be needed.
[2] Volumes are referring to total sample input, regardless of the use of preservative.

Note:

- Do not pre-mix the Binding Enhancer and Proteinase K.
- Do not change the order of pipetting.
- Once all components are added, proceed immediately to the instrument processing. There is no need for manual mixing beforehand.

2. Select the program on the instrument according to the following table.

For saliva input volume	Program
500 µL – 1 mL	**MagMAX_Ultra2_1mL_FLEX**
1.1 mL – 2 mL	**MagMAX_Ultra2_2mL_FLEX**

3. Start the run, and load the prepared plates into position when prompted by the instrument.

   During this on board Proteinase K sample digestion (~20 minutes) prepare the DNA Binding Bead Mix.

4 Purify the gDNA

1. Prepare DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate
500 µL – 1 mL saliva input (24 samples per plate)		
Binding Solution	1000 µL	26.40 mL
DNA Binding Beads	100 µL	2.64 mL
Total volume	**1100 µL**	**29.04 mL**
1.1 mL – 2 mL saliva input (24 samples per plate)		
Binding Solution	2000 µL	52.80 mL
DNA Binding Beads	200 µL	5.28 mL
Total volume	**2200 µL**	**58.08 mL**

2. When instructed by the instrument (~20 minutes after the run has started), remove the Sample Plate and add DNA Binding Bead Mix to each sample, according to the following table.

For saliva input volume	Add DNA Binding Bead Mix
500 µL – 1 mL	1100 µL
1.1 mL – 2 mL	2200 µL

Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

3. Immediately place the plate back onto the KingFisher™ Flex and follow the prompts on the instrument to allow the sample processing to proceed.

4. At the end of the run, immediately remove the plate from the instrument and transfer the eluate to the final tube/plate of choice for final storage.

The purified DNA is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Perform DNA purification using KingFisher™ Apex (large volume: 500 µL to 2 mL)

1 Set up the instrument

1. Ensure that the instrument is set up for processing with the proper magnetic head (24 deep-well) for your application.

2. Ensure that the proper heat block (24 well) is installed for your application.

3. Ensure that the proper program (**MagMAX_Ultra2_1mL** or **MagMAX_Ultra2_2mL**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plates

Set up the Wash, Elution, and Tip Comb Plates outside the instrument according to the following table.

Plate ID	Plate position	Plate type	Reagent	Volume per well
500 µL – 1 mL saliva input				
Wash I Solution Plate	3	Deep Well	Wash I Solution	2500 µL
Wash II Solution Plate 1	4	Deep Well	Wash II Solution	2500 µL
Wash II Solution Plate 2	5	Deep Well	Wash II Solution	1000 µL
Elution Plate	6	Deep Well	Elution Solution	150–200 µL
Tip Comb	1	KingFisher™ Flex 24 Deep-well Tip Comb and Plate		
1.1 mL – 2 mL saliva input				
Wash I Solution Plate	3	Deep Well	Wash I Solution	5000 µL
Wash II Solution Plate 1	4	Deep Well	Wash II Solution	4000 µL
Wash II Solution Plate 2	5	Deep Well	Wash II Solution	2000 µL
Elution Plate	6	Deep Well	Elution Solution	250–300 µL
Tip Comb	1	KingFisher™ Flex 24 Deep-well Tip Comb and Plate		

Note: The plates will be loaded onto the instrument immediately after the Sample Plate has been prepared.

3 Prepare Sample Plate and digest with Proteinase K

1. Transfer appropriate amount (according to the following table) of Enhancer Solution first, then sample, and lastly Proteinase K, to the appropriate wells of a deep-well plate. This is the Sample Plate.

Enhancer Solution (µL) [1]	Sample Volume (µL) [2]	Proteinase K (µL)
50	500	50
100	1000	100
200	2000	200

[1] If you are isolating from stabilized saliva, Enhancer Solution may not be needed.
[2] Volumes are referring to total sample input, regardless of the use of preservative.

Note:

- Do not pre-mix the Binding Enhancer and Proteinase K.
- Do not change the order of pipetting.
- Once all components are added, proceed immediately to the instrument processing. There is no need for manual mixing beforehand.

2. Select the program on the instrument according to the following table.

For saliva input volume	Program
500 µL – 1 mL	**MagMAX_Ultra2_1mL**
1.1 mL – 2 mL	**MagMAX_Ultra2_2mL**

3. Start the run, and load the prepared plates into position when prompted by the instrument.

   During this on board Proteinase K sample digestion (~20 minutes) prepare the DNA Binding Bead Mix.

4 Purify the gDNA

1. Prepare DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate
500 µL – 1 mL saliva input (24 samples per plate)		
Binding Solution	1000 µL	26.40 mL
DNA Binding Beads	100 µL	2.64 mL
Total volume	**1100 µL**	**29.04 mL**
1.1 mL – 2 mL saliva input (24 samples per plate)		
Binding Solution	2000 µL	52.80 mL
DNA Binding Beads	200 µL	5.28 mL
Total volume	**2200 µL**	**58.08 mL**

2. When instructed by the instrument (~20 minutes after the run has started), remove the Sample Plate and add DNA Binding Bead Mix to each sample, according to the following table.

For saliva input volume	Add DNA Binding Bead Mix
500 µL – 1 mL	1100 µL
1.1 mL – 2 mL	2200 µL

Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

3. Immediately place the plate back onto the KingFisher™ Apex and follow the prompts on the instrument to allow the sample processing to proceed.

4. At the end of the run, immediately remove the plate from the instrument and transfer the eluate to the final tube/plate of choice for final storage.

The purified DNA is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Perform DNA purification using KingFisher™ Duo Prime (small volume: 100–400 µL)

1 Set up the instrument

1. Ensure that the instrument is set up for processing with the proper magnetic head (12 pin) and heat block for your application.
2. Ensure that the proper program (**MagMAX_Ultra2_200µL_DUO** or **MagMAX_Ultra2_400µL_DUO**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plate

Prepare the plate and elution strip according to the following table. The Sample Row will be prepared in the next section.

Table 2 96 Deep-well plate layout (100–200 µL saliva input)

Row ID	Plate Row	Reagent	Volume per well
Plate layout			
Sample	A	Sample[1]	Varies
Tip Comb	B	Tip Comb	N/A
—	C	Empty	
Wash I Solution	D	Wash I Solution	500 µL
Wash II Solution	E	Wash II Solution	500 µL
Wash II Solution	F	Wash II Solution	500 µL
—	G	Empty	
—	H	Empty	
Duo elution strip			
Elution Solution	—	Elution Solution	50–100 µL

[1] See "Prepare Sample Row and digest with Proteinase K" on page 12.

Table 3 96 Deep-well plate layout (200–400 µL saliva input)

Row ID	Plate Row	Reagent	Volume per well
Plate layout			
Sample	A	Sample[1]	Varies
Tip Comb	B	Tip Comb	N/A
—	C	Empty	
Wash I Solution	D	Wash I Solution	1000 µL
Wash II Solution	E	Wash II Solution	1000 µL
Wash II Solution	F	Wash II Solution	500 µL
—	G	Empty	
—	H	Empty	
Duo elution strip			
Elution Solution	—	Elution Solution	50–100 µL

[1] See "Prepare Sample Row and digest with Proteinase K" on page 12.

Note: The plate and elution strip will be loaded onto the instrument immediately after the Sample Row has been prepared.

3 Prepare Sample Row and digest with Proteinase K

1. Transfer appropriate amount (according to the following table) of Enhancer Solution first, then sample, and lastly Proteinase K, to the appropriate wells (Row A) of the deep-well plate that was previously prepared.

Enhancer Solution (µL) [1]	Sample Volume (µL) [2]	Proteinase K (µL)
10	100	10
20	200	20
30	300	30
40	400	40

[1] If you are isolating from stabilized saliva, Enhancer Solution may not be needed.
[2] Volumes are referring to total sample input, regardless of the use of preservative.

Note:
- Do not pre-mix the Enhancer Solution and Proteinase K.
- Do not change the order of pipetting.
- Once all components are added, proceed immediately to the instrument processing. There is no need for manual mixing beforehand.

2. Select the program on the instrument according to the following table.

For saliva input volume	Program
100–200 µL	**MagMAX_Ultra2_200µL_DUO**
200–400 µL	**MagMAX_Ultra2_400µL_DUO**

3. Start the run, and load the prepared plate and elution strip into position when prompted by the instrument.

   During this on board Proteinase K sample digestion (~20 minutes) prepare the DNA Binding Bead Mix.

4 Purify the gDNA

1. Prepare DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate
100–200 µL saliva input (12 samples per plate)		
Binding Solution	200 µL	2.64 mL
DNA Binding Beads	20 µL	264 µL
Total volume	**220 µL**	**2.91 mL**
200–400 µL saliva input (12 samples per plate)		
Binding Solution	400 µL	5.28 mL
DNA Binding Beads	40 µL	528 µL
Total volume	**440 µL**	**5.81 mL**

2. When instructed by the instrument (~20 minutes after the run has started), remove Plate 1 and add DNA Binding Bead Mix to each sample (Row A), according to the following table.

For saliva input volume	Add DNA Binding Bead Mix
100–200 µL	220 µL
200–400 µL	440 µL

Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

4 *(continued)*

3. Immediately place the plate back onto the KingFisher™ Duo Prime and follow the prompts on the instrument to allow the sample processing to proceed.
4. At the end of the run, immediately remove the plate and elution strip from the instrument.
5. Seal the eluate within the strips with the dedicated caps and store, or transfer the eluate to a final tube/plate of choice for final storage.

The purified DNA is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Perform DNA purification using KingFisher™ Duo Prime (large volume: 500 µL to 2mL)

1 Set up the instrument

1. Ensure that the instrument is set up for processing with the proper magnetic head (6 pin) and heat block for your application.
2. Ensure that the proper program (**MagMAX_Ultra2_1mL_DUO** or **MagMAX_Ultra2_2mL_DUO**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plate

Prepare the 24DW plate according to the following tables.

Table 4 24DW plate layout (500 µL – 1 mL saliva input)

Row ID	Plate Row	Reagent	Volume per well
Plate 1 layout			
Sample	A	Sample[1]	Varies
Wash I Solution	B	Wash I Solution	2500 µL
Wash II Solution	C	Wash II Solution	2500 µL
Wash II Solution	D	Wash II Solution	1000 µL
Plate 2 layout			
Elution Solution	A	Elution Solution	150–200 µL
Tip Comb	B	Tip Comb	N/A
—	C	Empty	
—	D	Empty	

[1] See "Prepare Sample Row and digest with Proteinase K" on page 14.

Table 5 24DW plate layout (1.1 mL – 2 mL saliva input)

Row ID	Plate Row	Reagent	Volume per well
Plate 1 layout			
Sample	A	Sample[1]	Varies
Wash I Solution	B	Wash I Solution	5000 µL
Wash II Solution	C	Wash II Solution	4000 µL
Wash II Solution	D	Wash II Solution	2000 µL
Plate 2 layout			
Elution Solution	A	Elution Solution	250–300 µL
Tip Comb	B	Tip Comb	N/A
—	C	Empty	
—	D	Empty	

[1] See "Prepare Sample Row and digest with Proteinase K" on page 14.

2 *(continued)*

Note: The plates will be loaded onto the instrument immediately after the Sample Row has been prepared.

3 Prepare Sample Row and digest with Proteinase K

1. Transfer appropriate amount (according to the following table) of Enhancer Solution first, then sample, and lastly Proteinase K, to the appropriate wells (Row A) of the deep-well plate that was previously prepared.

Enhancer Solution (µL) [1]	Sample Volume (µL) [2]	Proteinase K (µL)
50	500	50
100	1000	100
200	2000	200

[1] If you are isolating from stabilized saliva, Enhancer Solution may not be needed.
[2] Volumes are referring to total sample input, regardless of the use of preservative.

Note:

- Do not pre-mix the Enhancer Solution and Proteinase K.
- Do not change the order of pipetting.
- Once all components are added, proceed immediately to the instrument processing. There is no need for manual mixing beforehand.

2. Select the program on the instrument according to the following table.

For saliva input volume	Program
500 µL – 1 mL	**MagMAX_Ultra2_1mL_DUO**
1.1 mL – 2 mL	**MagMAX_Ultra2_2mL_DUO**

3. Start the run, and load the prepared plates into position when prompted by the instrument.

   During this on board Proteinase K sample digestion (~20 minutes) prepare the DNA Binding Bead Mix.

4 Purify the gDNA

1. Prepare DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate
500 µL – 1 mL saliva input (6 samples per plate)		
Binding Solution	1000 µL	6.60 mL
DNA Binding Beads	100 µL	660 µL
Total volume	**1100 µL**	**7.26 mL**
1.1 mL – 2 mL saliva input (6 samples per plate)		
Binding Solution	2000 µL	13.20 mL
DNA Binding Beads	200 µL	1.32 mL
Total volume	**2200 µL**	**14.52 mL**

4 *(continued)*

2. When instructed by the instrument (~20 minutes after the run has started), remove Plate 1 and add DNA Binding Bead Mix to each sample (Row A), according to the following table.

For saliva input volume	Add DNA Binding Bead Mix
500 µL – 1 mL	1100 µL
1.1 mL – 2 mL	2200 µL

Note: Remix Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples/wells. Mixture is viscous, pipet slowly to ensure that the correct amount is added.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

3. Immediately place the plate back onto the KingFisher™ Duo Prime and follow the prompts on the instrument to allow the sample processing to proceed.

4. At the end of the run, immediately remove the plates from the instrument and transfer the eluate to the final tube/plate of choice for final storage.

The purified DNA is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

Quantitation

To most accurately quantitate gDNA samples isolated from saliva, it is recommended to quantitate using either the Qubit™ dsDNA BR (Broad Range) Assay Kit (Cat. No. Q32850) or Qubit™ dsDNA HS (High Sensitivity) Assay Kit (Cat. No. Q32851). Another acceptable method is quantitation utilizing qPCR and the Applied Biosystems™ TaqMan™ RNase P Detection Reagents Kit (Cat. No. 4316831).

Limited product warranty

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Revision	Date	Description
F	31 July 2024	Important statement added to the user guide under the section "Before first use of the kit".
E.0	12 April 2021	Support added for KingFisher™ Apex Purification System.
D.0	15 March 2019	Updated manufacturing address to Vilnius. Added note to address yellowing buffers and viscosity concerns.
C.0	10 January 2018	Correction of Flex small volume step 2 table tip comb entry.
B.0	6 December 2017	Addition of quantitation section, and minor edits.
A.0	8 September 2017	New document for MagMAX™ DNA Multi-Sample Ultra 2.0 Kit.