MagMAX™ Saliva gDNA Isolation Kit

High throughput isolation of gDNA from saliva

Catalog Numbers A39059, A39060

Pub. No. MAN0017722 **Rev.** A.0

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

Product description

The Applied Biosystems™ MagMAX™ Saliva gDNA Isolation Kit is developed for scalable, rapid purification of high-quality DNA from saliva, both preserved (saliva within a stabilizing reagent or a preservative) and fresh. You can use the DNA purified with this kit 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 and the KingFisher™ Duo Prime.

Contents and storage

Reagents that are provided in the kit are sufficient for 100 reactions (Cat. No. A39059) or 500 reactions (Cat. No. A39060) using small volume (≤500 µL) inputs.

Table 1 Components of MagMAX™ Saliva gDNA Isolation Kit

Component	100 reactions	500 reactions	Storage
Lysis/Binding Solution	55 mL	275 mL	15°C to 30°C
gDNA Binding Beads	4.5 mL	22 mL	
Wash I Solution	110 mL	2 × 275 mL	
Elution Solution	12 mL	60 mL	

For 1,000 reaction volume, use Cat. No. A39063 (Lysis/Binding Solution), A39061 (gDNA Binding Beads), A39062 (Wash I Solution), and A39064 (Elution Solution).

Required materials not supplied

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

Item	Source
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 with 96 Deep-Well Head	5400630
For large volume sample[2]: KingFisher™ Flex Magnetic Particle Processor with 24 Deep-Well Head	5400640
Equipment	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Consumables	
Deep-well plates:	
For small volume sample[1]: KingFisher™ Deepwell 96 Plate	95040450
For large volume sample[2]: KingFisher™ Flex 24 Deep-Well Plates	95040470
96-well standard plates (for use with KingFisher™ Flex only; tip comb placement and elution plate and/or 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™ deep-well 96 plate	97003500
KingFisher™ Duo Prime 6 tip comb, for use with the Flex 24 deep-well plate	97003510
KingFisher™ 96 tip comb for DW magnets, for Flex protocol only	97002534
KingFisher™ Flex 24 Deep Well Tip Comb and plate, for Flex protocol only	97002610
Materials	
MicroAmp™ Clear Adhesive Film	4306311

Item	Source
Reagents	
Ethanol, 96–100% (molecular biology grade)	MLS
Nuclease-free Water	AM9932

[1] Small volume saliva is ≤500 µL.
[2] Large volume saliva is 500 µL–2 mL.

General guidelines

- Perform all steps at room temperature (20–25°C), unless otherwise noted.
- Precipitates can occur if the Lysis/Binding Solution is stored when room temperature is too cold. If there are precipitates, warm the Lysis/Binding Solution at 37°C and gently mix to dissolve the precipitates. Avoid creating bubbles.
- Per-plate volumes for reagent mixes are sufficient for one plate plus overage. To calculate volumes for other sample numbers, see the per-well volume and add 10% overage.
- *(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.
- When isolating from preserved or stabilized saliva, verify with manufacturer recommendations before processing. Many saliva collection devices require an upfront incubation before sample processing can occur. Failure to do so can affect yields.

Guidelines for DNA Lysis/Binding Bead Mix

- Vortex gDNA Binding Beads thoroughly, then combine them with Lysis/Binding Solution in a nuclease-free tube and invert the tube until homogeneous. You can store this mixture 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.

Before each use of the kit

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

Perform DNA purification using KingFisher™ Flex (small volume: ≤500 µL)

1 Set up the instrument

a. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	96 deep-well magnetic head
Heat block	96 well standard heat block

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields.

b. Ensure that the proper program (**MagMAX_Saliva_500µL_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 saliva input				
Wash I Solution Plate	2	Deep-well	Wash I Solution	1,000 µL
Wash II Solution Plate 1	3	Deep-well	Wash II Solution	1,000 µL
Wash II Solution Plate 2	4	Deep-well	Wash II Solution	500 µL
Elution Plate	5	Standard	Elution Solution	50–100 µL
Tip Comb	6	Place a 96 Deep-well Tip Comb in a Standard Plate		

Note: Load the plates onto the instrument immediately after the Sample Plate has been prepared.

3 Prepare Sample Plate and purify the DNA

a. Prepare the DNA Lysis/Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate
≤500 µL saliva input (96 samples per plate)		
Lysis/Binding Solution	460 µL	48.58 mL
DNA Binding Beads	40 µL	4.22 mL
Total volume	**500 µL**	**52.80 mL**

b. Transfer the appropriate amount of the saliva sample to the appropriate wells of a new deep-well plate.

This plate is the Sample Plate.

c. Add 500 µL of the DNA Lysis/Binding Bead Mix to each sample in the Sample Plate.

Note:
- Remix the DNA Lysis/Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added.
- After you have added all components, proceed immediately to the instrument processing. There is no need for manual mixing beforehand.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

d. Select the program **MagMAX_Saliva_500µL_FLEX** on the instrument.

e. Start the run, then load the prepared plates into position when prompted by the instrument.

f. At the end of the run, immediately remove the plate from the instrument, then cover the plate or transfer the eluate to the final tube or 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

a. Ensure that the instrument is set up with the proper magnetic head and the proper heat block, as indicated in the following table.

Component	Type
Magnetic head	24 deep-well magnetic head
Heat block	24 well standard heat block

IMPORTANT! Failure to use the proper magnetic head and heat block results in lower yields.

b. Ensure that the proper program (**MagMAX_Saliva_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 and sample input volume.

Plate ID	Plate position	Plate type	Reagent	Volume per well
500 µL – 1 mL saliva input				
Wash I Solution Plate 1	2	Deep-well	Wash I Solution	1,000 µL
Wash I Solution Plate 2	3	Deep-well	Wash I Solution	1,000 µL
Wash II Solution Plate 1	4	Deep-well	Wash II Solution	2,000 µL
Wash II Solution Plate 2	5	Deep-well	Wash II Solution	500 µL
Elution Plate	6	Deep-well	Elution Solution	200 µL
Tip Comb	7	KingFisher™ Flex 24 Deep-well Tip Comb and Plate		
1 mL – 2 mL saliva input				
Wash I Solution Plate 1	2	Deep-well	Wash I Solution	2,000 µL
Wash I Solution Plate 2	3	Deep-well	Wash I Solution	2,000 µL
Wash II Solution Plate 1	4	Deep-well	Wash II Solution	2,000 µL
Wash II Solution Plate 2	5	Deep-well	Wash II Solution	500 µL
Elution Plate	6	Deep-well	Elution Solution	200 µL
Tip Comb	7	KingFisher™ Flex 24 Deep-well Tip Comb and Plate		

Note: Load the plates onto the instrument immediately after the Sample Plate has been prepared.

3 Prepare Sample Plate and purify the DNA

a. Prepare the DNA Lysis/Binding Bead Mix according to the following table and sample input volume.

Component	Volume per well	Volume per plate
500 µL – 1 mL saliva input (24 samples per plate)		
Lysis/Binding Solution	920 µL	24.29 mL
DNA Binding Beads	80 µL	2.11 mL
Total volume	**1,000 µL**	**26.40 mL**
1.1 mL – 2 mL saliva input (24 samples per plate)		
Lysis/Binding Solution	1,840 µL	48.58 mL
DNA Binding Beads	160 µL	4.22 mL
Total volume	**2,000 µL**	**52.80 mL**

b. Transfer the appropriate amount of the saliva sample to the appropriate wells of a new deep-well plate.

This plate is the Sample Plate.

c. Add DNA Lysis/Binding Bead Mix to each sample in the Sample Plate, according to the following table and sample input volume.

For saliva input volume	Add DNA Lysis/Binding Bead Mix
500 µL – 1 mL	1,000 µL
1.1 mL – 2 mL	2,000 µL

3 Prepare Sample Plate and purify the DNA *(continued)*

Note:

- Remix the DNA Lysis/Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added.
- After you have added all components, proceed immediately to the instrument processing. There is no need for manual mixing beforehand.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

d. Select the program **MagMAX_Saliva_2mL_FLEX** on the instrument.

e. Start the run, then load the prepared plates into position when prompted by the instrument.

f. At the end of the run, immediately remove the plate from the instrument, then cover the plate or transfer the eluate to the final tube or 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: ≤500 µL)

1 Set up the instrument

a. Ensure that the instrument is set up for processing with the proper magnetic head (12 pin) and heat block for your application.

b. Ensure that the proper program (**MagMAX_Saliva_500µL_DUO**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plate

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

Table 2 96 Deep-well plate layout (≤500 µL saliva input)

Row ID	Plate Row	Reagent	Volume per well
Elution Solution	A	Elution Solution	50–100 µL
—	B	Empty	
Tip Comb	C	Tip Comb	N/A
Wash II Solution row 2	D	Wash II Solution	500 µL
Wash II Solution row 1	E	Wash II Solution	1,000 µL
Wash I Solution	F	Wash I Solution	1,000 µL
—	G	Empty	
Sample	H	Sample	Varies

Note: Load the plate onto the instrument immediately after the Sample Row has been prepared.

3 Prepare Sample Row and purify the DNA

a. Prepare the DNA Lysis/Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate
≤500 µL saliva input (12 samples per plate)		
Lysis/Binding Solution	460 µL	6.07 mL
DNA Binding Beads	40 µL	528 µL
Total volume	**500 µL**	**6.60 mL**

b. Transfer the appropriate amount of the sample to the appropriate wells (Row H) of the deep-well plate that was previously prepared.

c. Add 500 µL of the DNA Lysis/Binding Bead Mix to each sample in Row H.

Note:

- Remix the DNA Lysis/Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added.
- After you have added all components, proceed immediately to the instrument processing. There is no need for manual mixing beforehand.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

d. Select the program **MagMAX_Saliva_500µL_DUO** on the instrument.

e. Start the run, then load the prepared plate into position when prompted by the instrument.

f. At the end of the run, immediately remove the plate from the instrument, then cover the plate or transfer the eluate to the final tube or plate of choice for final storage.

3 Prepare Sample Row and purify the DNA *(continued)*

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

a. Ensure that the instrument is set up for processing with the proper magnetic head (6 pin) and heat block for your application.

b. Ensure that the proper program (**MagMAX_Saliva_2mL_DUO**) has been downloaded from the product page and loaded onto the instrument.

2 Set up the processing plate

Prepare the 24 deep-well plates according to the following tables and sample input volume.

Table 3 24 Deep-well plate layout (500 µL – 1 mL saliva input)

Row ID	Plate Row	Reagent	Volume per well
Plate 1 layout			
Wash I Solution plate 2	A	Wash I Solution	1,000 µL
Wash I Solution plate 1	B	Wash I Solution	1,000 µL
Tip Comb	C	Tip Comb	N/A
Sample	D	Sample	Varies
Plate 2 layout			
Elution Solution	A	Elution Solution	200 µL
Empty	B	–	
Wash II Solution plate 2	C	Wash II Solution	500 µL
Wash II Solution plate 1	D	Wash II Solution	2,000 µL

Table 4 24 Deep-well plate layout (1 mL – 2 mL saliva input)

Row ID	Plate Row	Reagent	Volume per well
Plate 1 layout			
Wash I Solution plate 2	A	Wash I Solution	2,000 µL
Wash I Solution plate 1	B	Wash I Solution	2,000 µL
Tip Comb	C	Tip Comb	N/A
Sample	D	Sample	Varies
Plate 2 layout			
Elution Solution	A	Elution Solution	200 µL
Empty	B	–	
Wash II Solution plate 2	C	Wash II Solution	500 µL
Wash II Solution plate 1	D	Wash II Solution	2,000 µL

Note: Load the plates onto the instrument immediately after the Sample Row has been prepared.

3 Purify the DNA

a. Prepare the DNA Lysis/Binding Bead Mix according to the following table and sample input volume.

Component	Volume per well	Volume per plate
500 µL – 1 mL saliva input (6 samples per plate)		
Lysis/Binding Solution	920 µL	6.07 mL
DNA Binding Beads	80 µL	528 µL
Total volume	**1,000 µL**	**6.60 mL**
1.1 mL – 2 mL saliva input (6 samples per plate)		
Lysis/ Binding Solution	1,840 µL	12.14 mL
DNA Binding Beads	160 µL	1.06 mL
Total volume	**2,000 µL**	**13.20 mL**

b. Transfer the appropriate amount of the sample to the appropriate wells (Row D of Plate 1) of the deep-well plate that was previously prepared.

c. Add DNA Lysis/Binding Bead Mix to each sample in Row D of Plate 1, according to the following table.

For saliva input volume	Add DNA Lysis/Binding Bead Mix
500 µL – 1 mL	1,000 µL
1.1 mL – 2 mL	2,000 µL

Note:
- Remix the DNA Lysis/Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added.
- After you have added all components, proceed immediately to the instrument processing. There is no need for manual mixing beforehand.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

d. Select the program **MagMAX_Saliva_2mL_DUO** on the instrument.

e. Start the run, then load the prepared plates into position when prompted by the instrument.

f. At the end of the run, immediately remove the plate from the instrument, then cover the plate or transfer the eluate to the final tube or 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 that are 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 using qPCR and the Applied Biosystems™ TaqMan® RNase P Detection Reagents Kit (Cat. No. 4316831).

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Revision	Date	Description
A.0	10 May 2018	New document

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