MagMAX™ Saliva gDNA Isolation Kit

Manual isolation of gDNA from saliva

Catalog Numbers A39059, A39060

Pub. No. MAN0017773 **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 manual isolations using a plate format.

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
Equipment	
Plate shaker, capable of shaking plates at a minimum of 900 rpm	88880023
Magnetic stand-96	AM10027
Incubator with metal racks	MLS
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Analog Vortex Mixer	MLS
Consumables	
KingFisher™ Deepwell 96 Plate	95040450
KingFisher™ 96 KF microplate	97002540
Materials	
MicroAmp™ Clear Adhesive Film	4306311
Reagents	
Ethanol, 96–100% (molecular biology grade)	MLS
Nuclease-free Water	AM9932

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.
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. You can use the same MicroAmp™ Clear Adhesive Film throughout the procedure, unless it becomes contaminated.
- If using a plate shaker other than the recommended shaker, ensure that:
  a. The plate fits securely on the plate shaker.

b. The recommended speeds are compatible with the plate shaker. Ideal shaker speeds allow for thorough mixing without splashing.

- 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.
- 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.

Isolate DNA from saliva (50–400 µL)

1 Prepare Samples and add DNA Lysis/Binding Bead Mix

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

Component	Volume per well	Volume 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 deep-well plate.

c. Add 500 µL of the DNA Lysis/Binding Bead Mix to each sample, then mix well by pipetting up and down 5 times.

Note: Remix the DNA Lysis/Binding Bead Mix frequently during pipetting to ensure even distribution of beads to all sample or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

d. Seal the plate with MicroAmp™ Clear Adhesive Film, ensuring that it is adequately sealed around the individual wells.

e. Shake the sealed plate at 800 rpm (Setting 8) for 5 minutes.

f. Place the sealed plate on the magnetic stand for at least 5 minutes, or until all the beads have collected.

2 Wash the DNA Binding Beads

a. Keeping the plate on the magnet, carefully remove the cover and slowly aspirate, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

b. Remove the plate from the magnetic stand, then add 1 mL of Wash I Solution to each sample.

c. Reseal the plate, then shake at 800 rpm (Setting 8) for 1 minute.

d. Place the plate back on the magnetic stand for 1 minute, or until all the beads have collected.

2 Wash the DNA Binding Beads *(continued)*

e. Keeping the plate on the magnet, remove the cover carefully, then discard the supernatant from each well.

IMPORTANT! Avoid disturbing the beads.

f. Repeat step b–e using 1 mL of Wash II Solution.

g. Repeat step b–e using 500 µL of Wash II Solution.

h. Dry the beads by shaking the plate (uncovered) at 900 rpm (Setting 9) for 2 minutes.

3 Elute the DNA

a. Add 50–100 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.

b. Place the plate in an incubator at 70°C for 5 minutes.

c. Remove the plate from the incubator and place on the titer shaker at 800 rpm (Setting 8) for 5 minutes.

d. Place the sealed plate on the magnetic stand for 3 minutes to collect the beads against the magnets.

e. Keep the plate on the magnet and carefully remove the seal, then transfer the eluates (which contain the purified gDNA) to a fresh standard (not deep-well) plate.

IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately after the transfers are complete.

The purified DNA is ready for immediate use. Alternatively, store the plate at 2–6°C for 24 hours, or 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).

Limited product warranty

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Revision history: Pub. No. MAN0017773

Revision	Date	Description
A.0	10 May 2018	New document