MagMAX™ DNA Multi-Sample Ultra 2.0 Kit

Manual extraction of DNA from buccal swabs, buffy coat, saliva, or whole blood

Catalog Number A36570

Pub. No. MAN0017326 Rev. E

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 you through manual isolations using a plate format.

Contents and storage

Reagents provided in the kit are sufficient for 100 reactions.

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 1000 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.

Item	Source
Consumables	
KingFisher™ deep-well 96 plate	95040450
KingFisher™ 96 KF plate	97002540
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
Reagents	
Ethanol, 96–100% (molecular biology grade)	MLS
Nuclease-Free Water	AM9932
Phosphate Buffered Saline (PBS (1X), pH 7.4)	10010023
(Optional) for buffy coat only: MagMAX™ DNA multi-sample Ultra 2.0 Elution Solution	A36582
Materials	
MicroAmp™ Clear Adhesive Film	4306311
4N6FLOQSwabs™, regular tip	4473979

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
- Cover the plate during the incubation and shaking steps to prevent spill-over and cross-contamination. The same MicroAmp™ Clear Adhesive Film can be used throughout the procedure, unless it becomes contaminated.
- If using a plate shaker other than the recommended shaker, verify 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, refer to the per-well volume and add 10% overage.
- *For buccal swabs only*:
  a. When a higher concentration of DNA is required, two swabs may be processed in one well.
  b. Buccal swabs can be stored for up to 3 weeks at –20°C to 20°C before isolation.
  c. Equilibrate buccal swabs to room temperature to maximize DNA recovery.
- *For saliva only*: When isolating from a preserved saliva sample (saliva within a stabilizing reagent or a preservative) Enhancer Solution may need to be omitted from the workflow.
- *For buffy coat and whole blood only:* The MagMAX™ DNA Multi-Sample Ultra 2.0 Kit is compatible with samples that are collected in K_2EDTA, K_3EDTA, Streck DNA, Streck RNA, and Sodium Citrate Blood Collection Tubes (BCT).

  IMPORTANT! Do not collect blood in Sodium Heparin BCTs as the heparin used as the anticoagulant in these tubes inhibits downstream applications.
- *For buffy coat only:* One kit is sufficient to process 40–60 buffy coat samples at 50–200 µL input volume. To process up to 200 buffy coat samples, purchase additional Elution Solution (A36582).

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 DNA Binding Beads thoroughly, then combine them with Binding Solution in a nuclease-free tube and invert tube until homogenous. This mixture may be stored for up to 1 day before use.
- Ensure 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.

Isolate DNA from buccal swabs

1 Sample collection and storage

IMPORTANT! Use the recommended swab with a foam tip. Use of cotton or generic polyester swabs may result in lower DNA yields or DNA that contains PCR inhibitors.

1. Have test subjects thoroughly rinse their mouths with water and swallow prior to swabbing.
2. Remove swab from packaging and thoroughly swab both cheeks of the test subject for 30 seconds each to maximize collection of buccal cells.
3. If necessary, store buccal swabs in the original pouch.

IMPORTANT! Do not store buccal swabs in plastic tubes. Bacterial growth in sealed plastic tubes can cause DNA degradation.

1 *(continued)*

Buccal swabs can be stored for up to 3 weeks at –20°C to 20°C before isolation.

2 Prepare samples and digest with Proteinase K

1. Place one swab, swab-head down, into a deep-well plate.
2. Break enough of the stick off the swabs so that the swabs sit in the wells without protruding.

   The recommended swabs have an easy break point, below the swab, that appears as a slight indentation in the stick portion of the swab.
3. Prepare sufficient Proteinase K Mix according to the following table, then gently invert or pipet up and down several times to thoroughly mix components.

Component [1]	Volume per well	Volume per plate (96 samples)
Enhancer Solution	40 µL	4.22 mL
PBS	400 µL	42.24 mL
Proteinase K	40 µL	4.22 mL
Total volume	**480 µL**	**50.68 mL**

[1] Pipet the components in the order they are listed in the table.

IMPORTANT! Only make enough Mix for immediate use. Mix is not stable for prolonged periods and will result in a reduction of DNA yield.

4. Add 480 µL of the Proteinase K Mix to each well containing a swab.

   Be careful to avoid touching the pipette tip to the swab when pipetting the Proteinase K Mix into the sample wells.
5. Seal the plate with MicroAmp™ Clear Adhesive Film, then shake the sealed plate at 900 rpm (setting 9) for 5 minutes.
6. Take the plate off the plate shaker, then incubate at 65°C for ≥ 20 minutes.

IMPORTANT! Arrange plates in the incubator to allow adequate flow around the plate wells to ensure that samples quickly reach and maintain the incubation temperature.

3 Add DNA Binding Beads

1. During Proteinase K digestion, prepare the DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate (96 wells)
Binding Solution	400 µL	42.24 mL
DNA Binding Beads	40 µL	4.22 mL
Total volume	**440 µL**	**46.46 mL**

2. After Proteinase K digestion, transfer the lysates to the corresponding wells of a new deep-well plate, then discard the buccal swabs.

To remove buccal swab from the lysate	Procedure
Transfer the lysate to a new plate.	1. Set a multi-channel pipettor to 480 µL and transfer one row at a time. 2. Ensure each well contains 420–480 µL after transfer.

3 *(continued)*

3. Add 440 µL of DNA Binding Bead Mix to each sample well and pipet up and down 5 times to mix.

   Note: Mixture is viscous, pipet slowly to ensure that the correct amount is added.

   IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

4. Seal the plate with MicroAmp™ Clear Adhesive Film, ensuring that it is adequately sealed around the individual wells.
5. Shake the sealed plate for 5 minutes at 800 rpm (Setting 8).
6. Place the sealed plate on the magnetic stand for at least 5 minutes, or until all of the beads have collected.

4 **Wash the DNA Binding Beads**

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

   IMPORTANT! Avoid disturbing the beads.

2. Remove the plate from the magnetic stand and add 1 mL of Wash I Solution to each sample.
3. Reseal the plate and shake for 1 minute at 800 rpm (Setting 8).
4. Place the plate back on the magnetic stand for 1 minute, or until all the beads have collected.
5. Keeping the plate on the magnet, remove the cover carefully and discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

6. Repeat step b–e using 1 mL of Wash II Solution.
7. Repeat step b–e using 500 µL of Wash II Solution.
8. Dry the beads by shaking the plate (uncovered) for 2 minutes at 900rpm (Setting 9).

5 **Elute the gDNA**

1. Add 50–100 µL of Elution Solution to each sample and seal the plate with MicroAmp™ Clear Adhesive Film.
2. Place the plate in an incubator at 70°C for 5 minutes.
3. Remove the plate from the incubator and place on the titer shaker for 5 minutes at 800 rpm (Setting 8).
4. Place the sealed plate on the magnetic stand for 3 minutes to pellet the beads against the magnets.
5. 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.

For quantitation methods, see “Quantitation” on page 8.

Isolate DNA from buffy coat (50–200 µL)

Use up to 200 µL of buffy coat sample or up to 2 mL of whole blood equivalent as starting material. Do not exceed 200 µL of buffy coat.

1 Prepare samples 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 sample wells of a deep-well plate.

Enhancer Solution (µL)	Sample Volume (µL)	Proteinase K (µL)
5	50	10
10	100	20
15	150	30
20	200	40

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

2. Seal the plate with MicroAmp™ Clear Adhesive Film, ensuring that it is adequately sealed around the individual wells.
3. Vigorously vortex for ≥30 seconds to completely mix Enhancer Solution, sample, and Proteinase K.

   Note: Creating bubbles during this mixing step will not interfere with downstream processing.
4. Incubate the sample plate at 65°C for ≥20 minutes.

2 Add DNA binding beads

1. During Proteinase K digestion, prepare the DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate (96 wells)
Binding Solution	400 µL	42.24 mL
DNA Binding Beads	40 µL	4.22 mL
Total volume	**440 µL**	**46.46 mL**

2. After the incubation, add 440 µL of DNA Binding Bead Mix to each sample well and pipet up and down 5 times to mix.

   Note: Mixture is viscous, pipet slowly to ensure that the correct amount is added.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

3. Seal the plate with MicroAmp™ Clear Adhesive Film, ensuring that it is adequately sealed around the individual wells.
4. Shake the sealed plate for 5 minutes at 800 rpm (Setting 8).
5. Place the sealed plate on the magnetic stand for at least 5 minutes, or until all the beads have collected.

3 Wash the DNA Binding Beads

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

IMPORTANT! Avoid disturbing the beads.

2. Remove the plate from the magnetic stand and add 1 mL of Wash I Solution to each sample.
3. Reseal the plate and shake for 1 minute at 800 rpm (Setting 8).

3 *(continued)*

4. Place the plate back on the magnetic stand for 1 minute, or until all the beads have collected.
5. Keeping the plate on the magnet, remove the cover carefully and discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

6. Repeat step b–e using 1 mL of Wash II Solution.
7. Repeat step b–e using 500 µL of Wash II Solution.
8. Dry the beads by shaking the plate (uncovered) for 2 minutes at 900rpm (Setting 9).

4 **Elute the gDNA**

1. Add 200–300 µL of Elution Solution to each sample and seal the plate with MicroAmp™ Clear Adhesive Film.
2. Place the plate in an incubator at 70°C for 5 minutes.
3. Remove the plate from the incubator and place on the titer shaker for 5 minutes at 800 rpm (Setting 8).
4. Place the sealed plate on the magnetic stand for 3 minutes to pellet the beads against the magnets.
5. 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.

For quantitation methods, see “Quantitation” on page 8.

Isolate DNA from saliva or whole blood (50–400 µL)

1 Prepare samples 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.

Enhancer Solution (µL)	Sample Volume (µL)	Proteinase K (µL)
5	50	5
10	100	10
15	150	15
20	200	20
25	250	25
30	300	30
35	350	35
40	400	40

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

2. Seal the plate with MicroAmp™ Clear Adhesive Film, ensuring that it is adequately sealed around the individual wells.
3. Vigorously vortex for ≥30 seconds to completely mix Enhancer Solution, sample, and Proteinase K.

   Note: Creating bubbles during this mixing step will not interfere with downstream processing.
4. Incubate the sample plate at 65°C for ≥20 minutes.

2 Add DNA binding beads

1. During Proteinase K digestion, prepare the DNA Binding Bead Mix according to the following table.

Component	Volume per well	Volume per plate (96 wells)
Binding Solution	400 µL	42.24 mL
DNA Binding Beads	40 µL	4.22 mL
Total volume	**440 µL**	**46.46 mL**

2. After the incubation, add 440 µL of DNA Binding Bead Mix to each sample well and pipet up and down 5 times to mix.

   Note: Mixture is viscous, pipet slowly to ensure that the correct amount is added.

IMPORTANT! Avoid creating bubbles during mixing and aliquoting.

3. Seal the plate with MicroAmp™ Clear Adhesive Film, ensuring that it is adequately sealed around the individual wells.
4. Shake the sealed plate for 5 minutes at 800 rpm (Setting 8).
5. Place the sealed plate on the magnetic stand for at least 5 minutes, or until all the beads have collected.

3 Wash the DNA Binding Beads

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

   IMPORTANT! Avoid disturbing the beads.

2. Remove the plate from the magnetic stand and add 1 mL of Wash I Solution to each sample.
3. Reseal the plate and shake for 1 minute at 800 rpm (Setting 8).
4. Place the plate back on the magnetic stand for 1 minute, or until all the beads have collected.
5. Keeping the plate on the magnet, remove the cover carefully and discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

6. Repeat step b–e using 1 mL of Wash II Solution.
7. Repeat step b–e using 500 µL of Wash II Solution.
8. Dry the beads by shaking the plate (uncovered) for 2 minutes at 900rpm (Setting 9).

4 Elute the gDNA

1. Add 50–100 µL of Elution Solution to each sample and seal the plate with MicroAmp™ Clear Adhesive Film.
2. Place the plate in an incubator at 70°C for 5 minutes.
3. Remove the plate from the incubator and place on the titer shaker for 5 minutes at 800 rpm (Setting 8).
4. Place the sealed plate on the magnetic stand for 3 minutes to pellet the beads against the magnets.
5. 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.

For quantitation methods, see “Quantitation” on page 8.

Quantitation

To most accurately quantitate gDNA samples isolated from buccal swabs and 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).

To most accurately quantitate gDNA samples isolated from buffy coat and whole blood, it is recommended to quantitate using a NanoDrop™ spectrophotometer. 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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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0017326 E

Revision	Date	Description
E	31 July 2024	Important statement added to the user guide under the section "Before first use of the kit".
D.0	11 December 2020	Reduced the buffy coat sample number per kit because more Elution Solution is needed per buffy coat reaction.
C.0	15 March 2019	Updated manufacturing address to Vilnius. Added note to address yellowing buffers and viscosity concerns.
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.