Isolation of DNA for Vaginal Microbiota Profiling Experiments

Optimized for vaginal samples using the MagMAX™ DNA Multi-Sample Ultra Kit

Pub. No. MAN0015935 **Rev.** A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Vaginal Microbiota Profiling Experiments Application Guide* (Pub. No. MAN0015669). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This document is intended as a benchtop reference for experienced users of the MagMAX™ DNA Multi-Sample Ultra Kit (Cat. Nos. A25597 and A25598). See the *Vaginal Microbiota Profiling Experiments Application Guide* (Pub. No. MAN0015669) for detailed instructions and troubleshooting.

Compatible sample collection systems or media

See the collection system or media documentation provided by the manufacturer for information on sample collection and storage.

Sample collection system / media	Source
ThinPrep™ Pap test	Hologic™
BD SurePath™ test	BD™
ESwab™[1,3]	Copan Diagnostics™
Aptima™ Vaginal Swab Transport Media (STM)[2]	Hologic™
M4™ MicroTest™[3]	Remel™
Affirm Ambient Temperature Transport System	BD™
BD ProbeTec™ Swab diluent Q^x	BD™

[1] If samples appear dense or cloudy or have been stored >48 hours, see the Troubleshooting section of *Vaginal Microbiota Profiling Experiments Application Guide* (Pub. No. MAN0015669).

[2] Samples require minor modifications to the DNA isolation procedure; see *Vaginal Microbiota Profiling Experiments Application Guide* (Pub. No. MAN0015669).

[3] Process samples within 48 hours of collection.

Before first use of the kit

- Prepare the Wash Solutions from the concentrates:
  - Add 25 mL of isopropanol to Wash Solution 1 Concentrate, mix, and store at room temperature.
  - Add 132 mL of ethanol to Wash Solution 2 Concentrate, mix, and store at room temperature.
- Reconstitute the zymolyase with 500 µL of the provided storage buffer (final concentration of 4 U/µL), vortex to mix, then store at –20°C.

  See the documentation provided with the zymolyase for more information.

Set up the sample layout

Set up the sample plate layout, which provides sample tracking from the 96-well plate used for DNA isolation to the 96-well sample plate *.csv file used for import into the OpenArray™ Sample Tracker Software.

Note: We recommend three technical replicates of each reaction.

Tool	Source	Description
`96-well Sample Plate 1.csv` template	On the computer on which the OpenArray™ Sample Tracker Software is installed: `C:\Program Files\Applied Biosytems\Sample Tracking Utility\examples`	Contains a sample layout tab.

Perform DNA extraction and elution

1 Concentrate the samples

a. Gently shake or swirl the sample contents to ensure thorough mixing of the sample.

b. Following the sample layout, transfer up to 1 mL of sample to the appropriate wells of a deep-well plate.

c. Seal the plate with a clear adhesive film, then centrifuge for 15 minutes at 2,250 × *g* to concentrate the samples.

d. After centrifugation, carefully remove and discard as much supernatant as possible without disturbing the pellet.

Note: You can leave up to 100 µL of supernatant, especially if there is no pellet.

2 Digest the samples with the Preliminary Digestion Mix

a. Prepare sufficient Preliminary Digestion Mix according to the following table.

IMPORTANT! Prepare the Preliminary Digestion Mix no more than 30 minutes before use and store on ice. Prolonged storage at room temperature can reduce its efficiency.

Component	Volume per well	Volume per plate
B-PER™ Bacterial Protein Extraction Reagent	185 µL	18.5 mL
Lysozyme Solution	10 µL	1 mL
Zymolyase solution (4 U/µL)	5 µL	0.5 mL
Total Preliminary Digestion Mix	**200 µL**	**20 mL**

b. Add 200 µL of Preliminary Digestion Mix to each sample well.

(*Optional*) Mix by pipetting up and down 5 to 10 times to disperse large pellets.

c. Seal the plate with a clear adhesive film, then shake for 2 minutes at 1,050 rpm.

d. Incubate the plate for 15 minutes at 65°C.

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.

During the incubation, prepare the PK Mix (next section).

3 Digest the samples with Proteinase K

a. Prepare sufficient PK Mix according to the following table, then invert several times to thoroughly mix components.

IMPORTANT! Prepare the PK Mix no more than 30 minutes before use and store at room temperature. Do not place PK Buffer or PK Mix on ice, to avoid precipitation.

Component	Volume per well	Volume per plate
Proteinase K	8 µL	0.8 mL
PK Buffer	42 µL	4.2 mL
Total PK Mix	**50 µL**	**5.0 mL**

b. When the incubation with Preliminary Digestion Mix is complete, add 50 µL of PK Mix to each sample well of the plate.

c. Seal the plate with a clear adhesive film, then shake the sealed plate for 2 minutes at 1,050 rpm.

d. Incubate for 15 minutes at 65°C.

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.

4 Set up the processing plates

a. While the samples are incubating at 65°C, set up the Wash, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	150 µL
Wash Plate 2	3	Deep Well	Wash Solution 2	150 µL
Wash Plate 3	4	Deep Well	Wash Solution 2	150 µL
Elution Plate[2]	5	Standard	DNA Elution Buffer 1	30 µL
Tip Comb	6	Deep Well	Place a tip comb in the plate.	

[1] Position on the instrument

[2] The instrument prompts the user to add DNA Elution Buffer 2 to the Elution Plate, after incubation with DNA Elution Buffer 1.

b. (*Optional*) To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.

5 Add Multi-Sample DNA Lysis Buffer, Bead/RNase A Mix, and isopropanol

a. (*Optional*) If condensation is present at the end of the 65°C incubation, briefly centrifuge the plate for 1–2 minutes at 1,500 × *g*.

b. Prepare sufficient Bead/RNase A Mix according to the following table.

IMPORTANT! Prepare the Bead/RNase A Mix no more than 1 hour before use and store on ice. Prolonged storage at room temperature can reduce its efficiency.

Vortex the DNA Binding Beads at moderate speed to form a uniform suspension before preparing the Bead/RNase A Mix.

Component	Volume per well	Volume per plate
DNA Binding Beads	16 µL	1.6 mL
RNase A	5 µL	0.5 mL
Nuclease-free Water	19 µL	1.9 mL
Total Bead/RNase A Mix	**40 µL**	**4.0 mL**

c. Add 125 µL of Multi-Sample DNA Lysis Buffer to each sample.

d. Vortex the Bead/RNase A Mix at moderate speed to ensure thorough mixing, then add 40 µL to each sample.

If you see that the beads in the Bead/RNase A Mix are settling, vortex the mix again briefly before continuing to pipette.

e. Add 200 µL of isopropanol to each sample, then proceed immediately to process the samples on the instrument (next section).

6 Process samples on the instrument

a. Select the program on the instrument.

- KingFisher™ Flex Magnetic Particle Processor: **A25597_Vaginal**
- MagMAX™ Express-96 Magnetic Particle Processor: **4413021_DW_blood**

b. Start the run, remove the temporary paraffin plate seals (if present), then load the prepared processing plates in their positions when prompted by the instrument.

c. Load the sample plate (containing lysate, isopropanol, and Bead/RNase A Mix) at position 1 when prompted by the instrument.

d. When prompted by the instrument (approximately 25 minutes after initial start):

1. Remove the Elution Plate from the instrument.

6 Process samples on the instrument *(continued)*

2. Add 30 µL of DNA Elution Buffer 2 to each sample well.

   IMPORTANT! Add DNA Elution Buffer 2 immediately after the prompt, to prevent excessive drying of any beads that are still captured on the Tip Comb.

3. Load the Elution Plate back onto the instrument, and press **Start**.

e. At the end of the run (approximately 30 minutes after initial start), remove the Elution Plate from the instrument and seal immediately with a new clear adhesive film.

- (*Optional*) Eluates can be transferred to a new storage plate after collection.
- If you see excessive bead residue in the wells, place the Elution Plate on the Magnetic Stand-96 to capture any residue prior to downstream use of the DNA.

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 samples are ready for immediate use. Alternatively, store the covered Elution Plate:

- At 2–6°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Revision history: Pub. No. MAN0015935

Revision	Date	Description
A.0	28 June 2016	New document.