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DNA isolation for Urinary Tract Microbiota Profiling Experiments

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

Pub. No. MAN0017751 **Rev.** A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Urinary Tract Microbiota Profiling Experiments Application Guide* (Pub. No. MAN0017750). 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). For detailed instructions and troubleshooting, see the *Urinary Tract Microbiota Profiling Experiments Application Guide* (Pub. No. MAN0017750).

Compatible sample collection and storage

Collect urine samples using BD™ Vacutainer® urine collection cups and tubes.

- Compatible urine samples:
  - Unstabilized urine that is collected in sterile containers (BD™ Cat. No. 364975)
  - Urine that is collected and stored in Urine Analysis (UA) tubes (BD™ Cat. No. 364992)
  - Urine that is collected and stored in Culture and Sensitivity (C&S) tubes (BD™ Cat. No. 364951)
- *(Optional)* Store samples according to the instructions provided with the collection container, or use the following storage conditions:
  - Store at 4°C for up to one week.
  - Store at –80°C for long-term storage. We recommend storing samples in smaller volumes to prevent multiple freeze/thaw cycles.

Before first use of the kit

- Prepare the Wash Solutions from the concentrates:
  - Add 25 mL of isopropanol to Wash Solution 1 Concentrate (from the kit). Mix the isopropanol and concentrate, then store at room temperature.
  - Add 132 mL of ethanol to Wash Solution 2 Concentrate (from the kit). Mix the ethanol and concentrate, then store at room temperature.
  - Add 70 mL of isopropanol to Wash Solution 1 Concentrate (separately purchased bottle, Cat. No. AM8504). Mix the isopropanol and concentrate, then 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.

  For more information, see the documentation provided with the zymolyase.

Set up the sample layout

The sample plate layout 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.

Set up the sample plate layout using the CSV file described in the following table.

Note: We recommend at least 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 Biosystems\Sample Tracking Utility\examples`	Contains a sample layout tab.

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Perform DNA extraction and elution

1 Concentrate the samples

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

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

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

d. **IMPORTANT!** There may not be an obvious pellet. If a pellet is visible, be careful not to disturb the pellet.

After centrifugation, carefully remove, then discard the supernatant.

1. Set a P1000 pipette (or similar) to 900 µL.

   Note: We recommend using a manual P1000 multichannel pipette. An electronic multichannel pipette can also be used at low speeds (<6 on Ranin pipettes).
2. Angle the pipette so that the pipette tips sit at the bend from square to conical in the plate well.
3. Carefully remove supernatant, then discard.
4. Repeat substep d2 and substep d3.
5. Visually inspect the samples to ensure that all urine has been removed.

   If > 30 µL of urine remains, repeat substep d2 and substep d3.

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.

c. Seal the plate with a clear adhesive film, then shake at 1,050 rpm for 3 minutes.

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 at 1,050 rpm for 3 minutes.

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	300 µL
Wash Plate 2	3	Deep Well	Wash Solution 1	300 µL
Wash Plate 3	4	Deep Well	Wash Solution 2	150 µL
Wash Plate 4	5	Deep Well	Wash Solution 2	150 µL
Elution Plate[2]	6	Standard	DNA Elution Buffer 1	30 µL
Tip Comb	7	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 at 1,500 × *g* for 1–2 minutes.

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. *(Optional)* Add 10 µL of TaqMan® Universal DNA Spike In Control (Xeno™ DNA control) to each sample.

Note: For more information about the Xeno™ DNA control, see *TaqMan® Universal DNA Spike In Control Product Information Sheet* (Pub. No. MAN0017852).

e. 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.

f. 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_UTM**

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 30 minutes after initial start):

1. Remove the Elution Plate from the instrument.
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, then press **Start**.

e. At the end of the run (approximately 5 minutes after the addition of DNA Elution Buffer 2), 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.

Related documentation

Document	Pub. No.
Urinary Tract Microbiota Profiling Experiments Application Guide	MAN0017750
TaqMan® Universal DNA Spike In Control Product Information Sheet	MAN0017852
OpenArray™ Sample Tracker Software Quick Reference	4460657

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  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision	Date	Description
A.0	09 August 2018	New document.

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