Isolation of DNA for Genotyping Experiments

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

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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**.

This document is intended as a benchtop reference for experienced users of the MagMAX™ DNA Multi-Sample Ultra Kit (Cat. nos. A25597 and A25598). Refer to the *MagMAX™ DNA Multi-Sample Ultra Kit (human buccal swabs) User Guide* (Pub. no. MAN0010293) for detailed instructions and troubleshooting.

Before first use of the kit: prepare Wash Solutions

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.

Before each use of the kit

- Preheat the incubator to 65°C.
- Download the OA_Genotyping_CalcSheet from **thermofisher.com/oaqrc**.

  The OA_Genotyping_CalcSheet provides sample tracking from the 96-well plate used for DNA isolation to the 96-well sample plate .csv file used for import into OpenArray™ Sample Tracker Software. It is designed for use with an adjustable pipettor for transfer of samples from the 96-well plate to the 384-well sample plate.

  The OA_Genotyping_CalcSheet also includes formulas for reagent mix setup, calculation of DNA concentration, and normalization of DNA concentration.
- Vortex DNA Binding Beads thoroughly, then combine with Nuclease-free Water according to the following table.

Component	Volume per well	Volume per plate
DNA Binding Beads	16 µL	1.6 mL
Nuclease-free Water	24 µL	2.4 mL
Total DNA Binding Bead Mix	40 µL	4 mL

Store DNA Binding Bead Mix at room temperature for up to 24 hours.

Set up the sample layout for the downstream OpenArray™ Sample Tracker Software and AccuFill™ step

Enter the sample layout for the 96-well DNA isolation plate into the OA_Genotyping_CalcSheet.

Digest the samples with Proteinase K

If swabs were frozen for storage, equilibrate to room temperature before starting this procedure.

Ensure that the incubator is preheated to 65°C.

1. Place the swab, swab-head down, in a well of a MagMAX™ Express-96 Deep Well Plate (one per well).

   If two swabs were collected, store the second swab as a backup sample.
2. Break enough of the stick off the swabs so that the swabs sit in the wells without protruding.
3. Prepare sufficient PK Mix according to the following table. Invert PK Mix several times to thoroughly mix components.

   IMPORTANT! Prepare the PK Mix just before use. Do not place PK Buffer or PK Mix on ice, to avoid precipitation.

Component	Volume per well	Volume per plate
Proteinase K	8 µL	800 µL
PK Buffer	192 µL	19.2 mL
Total PK Mix	200 µL	20 mL

4. Add 200 µL of PK Mix to each sample well of a MagMAX™ Express-96 Deep Well Plate (PK Plate).

   IMPORTANT! Do not touch any part of the swab with the pipet tip when pipetting the PK Mix in the sample wells.
5. Seal the plate with a MicroAmp™ Clear Adhesive Film and shake the sealed plate for 5 minutes at speed 8 (900–950 rpm).

   Ideal shaker speeds allow for thorough mixing without splashing.

6. Incubate for 20–45 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.

Set up the processing plates

1. While the samples are incubating, 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	50 µL
Tip Comb	6	Deep Well	Place a MagMAX™ Express-96 Deep Well Tip Comb in a MagMAX™ Express-96 Deep Well 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.

2. To prevent evaporation and contamination, cover the prepared processing plates with paraffin film until they are loaded into the instrument.

Add Multi-Sample DNA Lysis Buffer, isopropanol, and DNA Binding Bead Mix

1. (*Optional*) If condensation is present at the end of the 65°C incubation, briefly centrifuge the plate for 1–2 minutes at 1500 × *g*.

2. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample.

3. Seal the plate with a MicroAmp™ Clear Adhesive Film and shake for 5 minutes at speed 8 (900–950 rpm).

4. Transfer lysates to the wells of a new MagMAX™ Express-96 Deep Well Plate and discard the plate containing the buccal swabs.

5. Add 240 µL of isopropanol to each sample, seal the plate, and shake for 5 minutes at speed 8 (900–950 rpm).

6. Vortex the DNA Binding Bead Mix at moderate speed to ensure thorough mixing, add 40 µL to each sample, then proceed immediately to DNA isolation (next section).

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

Process samples on the instrument

1. Select the program on the instrument.
   - MagMAX™ Express-96 Magnetic Particle Processor: **4413021 DW blood**
   - KingFisher™ Flex Magnetic Particle Processor: **A25597_Blood_Buccal**

2. Start the run, remove the temporary paraffin plate seals, and load the prepared processing plates in their positions when prompted by the instrument (see "Set up the processing plates" on page 2).

3. Load the PK plate (containing lysate, isopropanol, and DNA Binding Bead Mix) at position 1 when prompted by the instrument.

4. When prompted by the instrument (approximately 28–30 minutes after initial start):
   a. Remove the Elution Plate from the instrument.
   b. Add 50 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.

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

5. At the end of the run (approximately 30–35 minutes after initial start), remove the Elution Plate from the instrument and seal immediately with a new MicroAmp™ Clear Adhesive Film.
   - *(Optional)* Eluates can be transferred to a new storage plate after collection.

   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.

The information in this guide is subject to change without notice.

For support visit **thermofisher.com/support** or email **techsupport@lifetech.com**

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