




Version 2 ▼

Jun 24, 2022

DNA Extraction Microbiome Kit - Fecal V.2

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**Stephanie Clouser**

ABSTRACT

DNA Extraction of fecal samples with the MagMAX Microbiome Kit from Applied Biosystems.

PROTOCOL CITATION

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<https://protocols.io/view/dna-extraction-microbiome-kit-fecal-cbynspve>
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MATERIALS TEXT

- a. MagMAX™ MICROBIOME Ultra Nucleic Acid Isolation Kit
- b. 200PF Molecular Grade Ethanol
- c. Nuclease-Free Water
- d. Sterile Pipette Tips (P100, P1000)
- e. Serological Pipettor
- f. Serological Pipettes (25, 50)
- g. 50 mL conical tube(s)
- h. Plate Tube rack
- i. Reagent Reservoirs
- j. 5 Deep-Well Plates
- k. 2 Elution Plate
- l. 1 Tip comb
- m. Plate Films
- n. Bead tubes
- o. Zymo 1:10 (for positive control)
- p. VWR marker
- q. Scissors
- r. Plate Map (reference "2.3_Plate-Map-Template")

Before You Begin

20m

1

Prepare your workspace

15m

- a. Turn on Biological Safety Cabinet blower, white light, and window alarm.
- b. Clean the cabinet with 70% ethanol, including the work surface, walls, and glass.
- c. Lower the sash and run the UV light for 15 minutes.



Do not trust the glass to protect you from UV exposure. You can be in a different part of the lab while it is running, but do not loiter in front of the cabinet.

- d. Switch the UV light back to white light; you are ready to begin.
- e. Clean all materials with 70% ethanol before putting them into the cabinet.

2

Gather Materials


5m

You can use the small projector cart to hold materials and your notes next to the cabinet. Clean cart well with 70% ethanol before use.

- a. MagMAX™ MICROBIOME Ultra Nucleic Acid Isolation Kit
- b. 200PF Molecular Grade Ethanol
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- e. Serological Pipettor
- f. Serological Pipettes (25, 50)
- g. 50 mL conical tube(s)
- h. Plate Tube rack
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- j. 5 Deep-Well Plates


- k. 2 Elution Plate
- l. 1 Tip comb
- m. Plate Films
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Lyse Sample 1h

- 3
1. Add  **800 µL** of Lysis Buffer to bead tubes.
 2. Prepare fecal samples as follows:

2m

A	B
Fresh/Frozen Fecal Samples	Weigh out 100mg, then place into prepared bead tubes.
Fecal samples in storage solution	Remove 200uL, then place into prepared bead tubes.
Fecal Swabs	Remove the plastic stick, then place swab into prepared bead tubes.

3. Place tubes in bead ruptor for 10 minutes at 20 Hz.
4. Remove tubes from bead ruptor and centrifuge.  **12500 rpm, 25°C, 00:02:00**
 - a. At this point bead tubes can be stored at 4°C overnight or at -20°C for up to 3 months.

Prepare Plates 1h

4

1h 30m

A	B	C	D
Plate ID	Plate Type	Reagent	Volume Per Well
Tip Comb	Standard	Place a 96 deep-well tip comb on a standard plate.	
Sample Plate	Deep Well	Sample + Binding Bead Mix as described in the protocol.	
Wash 1	Deep Well	Wash Buffer	1000uL
Wash 2	Deep Well	Wash Buffer	1000uL
Wash 3	Deep Well	80% Ethanol	1000uL
Wash 4	Deep Well	80% Ethanol	1000uL
Elution	Standard	Elution Buffer	200uL*

*Can be reduced to 100uL if using 1 fecal swab

1. Prepare mixture of Viral/Pathogen Binding Solution + DNA/RNA Binding Beads
 - a. Combine **500uL/sample of Viral/Pathogen Binding Solution** and **20uL/sample of DNA/RNA Binding Beads.**



The Viral/Pathogen Binding Solution is very viscous!

- i. Prepare enough for 100rxns if running a full plate.
- ii. Mix solution well by inversion and store at room temperature. Do not vortex or it will be too foamy to aliquot.



A	B
<i>In each of two 50mL conical tubes (50 rxns each):</i>	
Viral/Pathogen Binding Solution	25mL
DNA/RNA Binding Beads (vortex vigorously first)	1mL (or 1000uL)

2. Prepare 80% Ethanol Wash Buffer

- a. Make enough 80% ethanol for 2mL per sample. Check the shelf of kit reagents for leftover 80% ethanol in the labeled flask.

A	B
In a clean flask (100rxns total):	
100% molecular grade ethanol (in flammable cabinet)	160mL
Invitrogen Ultra-Pure Water	40mL

3. Prepare Sample Plate

- a. Remove tubes from bead beater.
- b. Transfer  **400 µL** of sample to appropriate well of a deep-well plate.
- c. Invert Bead Binding Mix, then add  **520 µL** to each sample in the sample plate.



The Bead Binding Mix is very viscous. Use a fresh pipette tip for every well.

- d. Proceed directly to the KingFisher.

KingFisher Processing

35m

5

1. Turn on the KingFisher Flex (the on switch is on the bottom left of the machine).
2. Push the right arrow to select "user" then the down arrow to select "DNA."
3. Push the down arrow until you reach "MagMAX_Microbiome_Stool_Flex" and then press "Start."
4. Load your prepared plates onto the machine following the prompts on the screen. Press the "Start" button to

35m

advance to the next steps.

a. BE SURE THAT THE PLATE IS ORIENTED CORRECTLY, with the A1 corner of the plate. matching the A1 labeled

on the KingFisher plate dock. Make sure the plate is sitting perfectly flat and not at a slight angle.

5. Once all plates are loaded, press "Start" to begin the protocol. Record usage in the logbook next to the machine.

6. After the program completes, you will be prompted to unload your plates the same way you loaded them. Keep the

Elution plate, seal with fresh plate film, and store at 4C for short term.



If you are storing for longterm, transfer the DNA from the Elution plate to non-stick DNA tubes and store in -80C.

7. All other plates can be discarded in the biohazard waste bin.

8. Turn off the KingFisher and wipe with DNA away.