Jun 23, 2022

DNA Extraction Microbiome Kit - Fecal

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This protocol is published without a DOI.

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ABSTRACT

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

PROTOCOL CITATION

Stephanie Clouser 2022. DNA Extraction Microbiome Kit - Fecal. **protocols.io** https://protocols.io/view/dna-extraction-microbiome-kit-fecal-cbvysn7w

CREATED

Jun 23, 2022

LAST MODIFIED

Jun 23, 2022

PROTOCOL INTEGER ID

65176

Before You Begin 20m

1 15m

Prepare your workspace

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.
i. 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 5m

Gather Materials

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
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”)

Lyse Sample 1h

3 1h

1. Add 800 uL of Lysis Buffer to bead tubes.
2. Prepare fecal samples as follows:

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. Vortex the bead tubes upside down for 10 seconds.
4. Place tubes in bead ruptor for 10 minutes at 20 Hz.
    a. At this point bead tubes can be stored at 4°C overnight.

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. Note: These solutions are 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
In each of two 50mL conical tubes (50 rxns each):	
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 400uL of sample to appropriate well of a deep-well plate.
        i. at this point, the lysate can be stored at -20C for 3 months.

c. Invert Bead Binding Mix, then add 520uL to each sample in the sample plate.
d. Proceed directly to the KingFisher.

KingFisher Processing 35m

5 35m

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
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.