**BEFORE YOU BEGIN**

1. **Prepare your workspace**
   1. Turn on Biological Safety Cabinet blower, white light, and window alarm.
   2. Clean the cabinet with 70% ethanol, including the work surface, walls, and glass.
   3. Lower the sash and run the UV light for 15 minutes.
      1. 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.
   4. Switch the UV light back to white light; you are ready to begin.
   5. Clean all materials with 70% ethanol before putting them into the cabinet.
2. **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.

* 1. MagMAX™ MICROBIOME Ultra Nucleic Acid Isolation Kit
  2. 200PF Molecular Grade Ethanol
  3. Nuclease-Free Water
  4. Sterile Pipette Tips (P100, P1000)
  5. Serological Pipettor
  6. Serological Pipettes (25, 50)
  7. 50 mL conical tube(s)
  8. Plate Tube rack
  9. Reagent Reservoirs
  10. Bead tubes
  11. 2.0mL microcentrifuge tubes
  12. Zymo 1:10 (for positive control)
  13. Tube rack magnet
  14. Plate Map (reference “2.3\_Plate-Map-Template”)

**LYSE SAMPLE**

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

|  |  |
| --- | --- |
| Bacteria/Yeast culture | Remove 500uL of the prepared culture, and then place in the prepared bead tube. |
| Soil Samples | Weigh out 200-250mg and place in tube. |
| Biofluid or Liquid Sample | Remove 400-500uL and place in tube. |
| Skin/Buccal Swab | Remove plastic stick and place in tube. |

1. Vortex the bead tubes upside down for 10 seconds.
2. Place tubes in bead ruptor for 10 minutes at 20 Hz. Remove and centrifuge for 2 minutes at 14,000xg
   1. At this point bead tubes can be stored at 4°C overnight.

**PREPARE REAGENTS**

1. Prepare mixture of Viral/Pathogen Binding Solution + DNA/RNA Binding Beads
   1. Combine 500 ul/sample of Viral/Pathogen Binding Solution and 20 ul/sample of DNA/RNA Binding Beads. Note: these solutions are very viscous!
      1. Prepare enough for 100 rxns if running a full plate:
      2. Mix solution well by inversion and store at room temperature. Do not vortex, or it will be too foamy to aliquot.

|  |  |
| --- | --- |
| *In each of two 50 mL conical tubes (50 rxns each)* | |
| **Viral/Pathogen Binding Solution** | **25 mL** |
| **DNA/RNA Binding Beads**  *(vortex vigorously first)* | **1 mL (or 1000 ul)** |

1. Prepare 80% Ethanol Wash Buffer
   1. Make enough 80% Ethanol for 2 mL per sample. Check the shelf to the right of the EpMotion for leftover 80% Ethanol in a labeled flask.

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

**DIGEST WITH PROTEINASE K**

* + - 1. Transfer 400-500uL of sample to a new 2.0mL microcentrifuge tube.
      2. Add 40uL of Proteinase K to each sample.
      3. Vortex the samples for 5 minutes.
      4. Incubate the samples in incubator or water bath at 65C for 20 minutes.

**BIND BEADS**

1. Transfer up to 400uL of the sample to a new clean 2.0mL microcentrifuge tube.
2. Add 520uL of binding bead mix to each sample.
   1. Make sure to use a new pipette tip for every sample, this solution is very viscous.
3. Vortex the tubes for 5 minutes.
4. Place tubes on magnet rack for at least 5 minutes, or until all the beads have collected.

**WASH BEADS**

* + - 1. Keeping tubes on the magnet, remove and discard the supernatant.
      2. Remove plate from magnet, add 1mL of Wash Buffer to each sample. Pipette to mix.
      3. Place plate back on magnet stand for 3 minutes, or until all the beads have collected.
      4. Keeping the tubes on the magnet, remove and discard the supernatant.
      5. Repeat steps 2-4 with 1mL of Wash Buffer.
      6. Repeat steps 2-4 with 1mL of 80% Ethanol.
      7. Repeat steps 2-4 with 1mL of 80% Ethanol.
      8. Dry the beads by leaving the tubes open and on the magnet for 2 minutes.

**ELUTE DNA**

Remove the tubes from the magnet rack, and add 50uL of Elution Buffer to each sample.

Place the tubes in an incubator or water bath at 75C for 5 minutes.

Place tubes on vortexer for 5 minutes.

Place the tubes on the magnet rack for 3 minutes or until all beads have collected.

Transfer supernatant to a new tube, properly labeled for each sample. This is your eluted DNA and can be stored at -80C for long term storage.