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MagMAX™ CORE Nucleic Acid Purification Kit

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We use this protocol and it's working

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Abstract

MagMAX™ CORE Nucleic Acid Purification Kit

1

Prepare the clarified lysate

1. Prepare samples according to sample type.

For...	Do this...
Environmental samples Feces	1. Transfer 0.2-0.3 g of sample to a 2-mL tube. 2. Add 1 mL of PBS, pH 7.4, then vortex vigorously for 3 minutes. 3. Centrifuge as indicated. • **For viral nucleic acid purification**—centrifuge at 15,000 × *g* for 1 minute. • **For bacterial DNA purification or concurrent purification of bacterial and viral nucleic acids**—centrifuge at 100 × *g* for 1 minute. 4. Proceed with 200 µL of supernatant.

For step 3 in box above, follow bacterial DNA purification centrifuge step.

2

2. Add Lysis Solution, then clarify the lysate.

For...	Do this...
Processing in tubes	1. For each sample, add 450 µL of Lysis Solution to a new 2-mL tube. 2. Add the indicated volume of sample from step 1 on page 19 to the Lysis Solution. 3. Vortex vigorously for 3 minutes. 4. Centrifuge at 15,000 × *g* for 2 minutes. 5. Remove the supernatant (clarified lysate) without disturbing the pellet.

For step 2 in box above, add 200 µl of supernatant from step 1 to the Lysis solution

3 Select
the programme 'MagMAXCORE_KF96_kf2 on the MagMAX machine (I used MagMAX express 96 particle processor machine)

4

Set up the processing plates

1. Set up the processing plates.

Table 9 Plate setup: KingFisher™ Flex or MagMAX™ Express-96 instrument

Plate ID	Plate position[1]	Plate type	Reagent	Volume per well
Wash Plate 1	2	Deep Well	MagMAX™ CORE Wash Solution 1	500 µL
Wash Plate 2	3	Deep Well	MagMAX™ CORE Wash Solution 2	500 µL
Elution	4	Standard	MagMAX™ CORE Elution Buffer	90 µL
Tip Comb	5	Standard	Place a tip comb in the plate.	

[1] Position on the instrument.

As per the table above, first get your plates ready before you load them onto the machine. You will need: 2 deep well plates for the wash solutions. LABEL THE PLATES on the side so you know which plate is which and then add in 500 µl of the appropriate wash buffer solution to the amount of columns you will be using (8 samples = one column etc. make sure to fill up a whole column as this is preferable to avoid damaging the machine). If you don't have enough samples to make up a full column, fill the empty wells with water. The elution plate is a normal (shallow plate). Make sure to label this on the side too and add 90ul of elution buffer to the amount of columns you will be using.

5 Note that the 'tip comb' plate 5 is the empty tip combs placed in an empty standard (shallow) plate. This is placed into the machine first (the screen on the machine tells you which plate to load in – starts on plate 5 and works backwards). When loading in your plates, make sure the A1 of the plate lines up with the A1 indicated in the plate holder of the machine. When placed in, the plates can 'bounce' when pressed in lightly. There is no clicking sound or fast securing of the plates in the machine so no need to use force. Close the door of the machine each time you've loaded in your plate then press 'start' and the machine will move the rotator around so you can place your next plate in (which will also be displayed on the machines screen). Remember you add the plates in the following order: plate 5,4,3,2,1, sample plate.

6 You will also need to make up your 'sample' plate, which is the plate to be added into the machine last. You will need to make a PK/bead mix to go into this sample plate. Make this by:

Prepare Bead/PK Mix

We recommend that you prepare new Bead/PK Mix for each processing run. If necessary, you can store Bead/PK Mix at 4°C for up to 1 week.

1. Vortex the MagMAX™ CORE Magnetic Beads thoroughly to ensure that the beads are fully resuspended.
2. Combine the following components for the required number of samples plus 10% overage.

Component	Volume per sample
MagMAX™ CORE Magnetic Beads	20 µL
MagMAX™ CORE Proteinase K	10 µL
Total Bead/PK Mix	**30 µL**

7 Set up the sample plate (**in a deep well plate**) as indicated below:

Combine the clarified lysate with Bead/PK Mix and MagMAX™ CORE Binding Solution

1. Invert the tube of Bead/PK Mix several times to resuspend the beads, then add 30 µL of the Bead/PK Mix to the required wells in the plate or tube strip.
2. Transfer the appropriate volume of each clarified lysate (see "Prepare the clarified lysate" on page 19) to a well with the Bead/PK Mix.

For...	Use...
Oral fluid	600 µL
Environmental samples, fecal samples, and swabs	500 µL

3. Mix the sample with Bead/PK Mix for 2 minutes at room temperature according to your mixing method.
   - **Using a plate shaker**—Shake vigorously for 2 minutes (see "Determine the maximum plate shaker setting" on page 9).
   - **By pipetting**—Pipet up and down several times, then incubate for 2 minutes at room temperature. (For downstream processing on the KingFisher™ mL Magnetic Particle Processor, you must mix by pipetting.)
4. Add 350 µL of MagMAX™ CORE Binding Solution.
5. Immediately proceed to process samples on the instrument (next section).

8 Once all your plates are loaded in, press start and the machine will immediately start up – you will notice that the machine will grab the tip combs from the plate five position and then swing back to begin working on plate one (your sample plate) and move from plate one to plate four (elution plate). The run takes less ~1 hour. When the run is complete the machine screen will just say 'paused'. Press stop and then remove all plates by pressing the left and right arrows (or

the start button). The elution plate wont have a cover on it so you may wish to place a seal or some parafilm over it.

9 Additional bead clean up step to remove potential trace magnetic beads leftover from machine - place the elution plate on a magnetic stand for 2 minutes and then take up the eluate from the centre (as beads pulled to the side of the well). The elution volume for the MagMAX is 90 µl but I got back 75-80 µl after bead clean up.

10 Store at -70/-80°C until further use.