3 Extract RNA (manual method)

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Manual RNA extraction can be performed from a sample input volume of 200 µL or 400 µL using either the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit.

Before you begin

- Determine the number of required reactions based on the number of patient samples to be processed, plus one Negative Control per plate.
- Prepare fresh 80% Ethanol using Ethanol, Absolute, Molecular Biology Grade and Nuclease-free Water (not DEPC-Treated) for the required number of reactions, plus 10% overage.

Sample input volume	Volume of 80% Ethanol per reaction
200 µL	0.75 mL
400 µL	1.5 mL

- Mark the Negative Control well on the plate.

Extract RNA—Manual method (200-µL sample input volume)

The following procedure uses components from the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit.

Prepare Binding Bead Mix (200-µL sample input volume)

Prepare the required amount of Binding Bead Mix on each day of use.

1. Vortex the Total Nucleic Acid Magnetic Beads to ensure that the bead mixture is homogeneous.
2. For the number of required reactions, prepare the Binding Bead Mix according to the following table:

Component	Volume per well[1]
Binding Solution	265 µL
Total Nucleic Acid Magnetic Beads	10 µL
Total volume per well	275 µL

[1] Include 10% overage when making the Binding Bead Mix for use with multiple reactions.

3. Mix well by inversion, then store at room temperature.

Digest with Proteinase K (200-µL sample input volume)

1. Add 5 µL of Proteinase K to each well of a KingFisher™ Deepwell 96 Plate.
2. Add 200 µL of sample to each sample well.
3. Add 200 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Invert the Binding Bead Mix 5 times gently to mix, then add 275 µL to each sample well and Negative Control well.

   Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The Binding Bead Mix is viscous, so pipet slowly to ensure that the correct amount is added. DO NOT reuse pipette tips to add Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.

5. Add 5 µL of MS2 Phage Control to each sample well and to the Negative Control well.
6. Seal the plate with MicroAmp™ Clear Adhesive Film, then shake the sealed plate at 1,050 rpm for 2 minutes.
7. Incubate the sealed plate at 65°C for 5 minutes (ensure the bottom of the plate is uncovered), then shake the plate at 1,050 rpm for 5 minutes.
8. Place the sealed plate on the magnetic stand for 10 minutes or until all of the beads have collected.

Wash the beads (200-µL sample input volume)

1. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

2. Remove the plate from the magnetic stand, then add 500 µL of Wash Buffer to each sample.
3. Reseal the plate, then shake at 1,050 rpm for 1 minute.
4. Place the plate back on the magnetic stand for 2 minutes, or until all the beads have collected.
5. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

6. Repeat step 2 to step 5 using 500 µL of 80% Ethanol.
7. Repeat step 2 to step 5 using 250 µL of 80% Ethanol.
8. Dry the beads by shaking the plate (uncovered) at 1,050 rpm for 2 minutes.

Elute the nucleic acid (200-µL sample input volume)

1. Add 50 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.
2. Shake the sealed plate at 1,050 rpm for 5 minutes.
3. Place the plate in an incubator at 65°C for 10 minutes.
4. Remove the plate from the incubator, then shake the plate at 1,050 rpm for 5 minutes.
5. Place the sealed plate on the magnetic stand for 3 minutes or until clear to collect the beads against the magnets.
6. Keeping the plate on the magnet, carefully remove the seal, transfer the eluates to a fresh standard (not deep-well) 96-well plate, then seal the plate with MicroAmp™ Clear Adhesive Film.

   IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately after the transfers are complete.

Note: Significant bead carry over may adversely impact RT-PCR performance.

Place the plate on ice for immediate use in real-time RT-PCR.

Extract RNA—Manual method (400-µL sample input volume)

The following procedure uses components from the MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit or the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit.

Prepare Binding Bead Mix (400-µL sample input volume)

Prepare the required amount of Binding Bead Mix on each day of use.

1. Vortex the Total Nucleic Acid Magnetic Beads to ensure that the bead mixture is homogeneous.
2. For the number of required reactions, prepare the Binding Bead Mix according to the following table:

Component	Volume per well[1]
Binding Solution	530 µL
Total Nucleic Acid Magnetic Beads	20 µL
Total volume per well	550 µL

[1] Include 10% overage when making the Binding Bead Mix for use with multiple reactions.

3. Mix well by inversion, then store at room temperature.

Digest with Proteinase K (400-µL sample input volume)

1. Add 10 µL of Proteinase K to each well of a KingFisher™ Deepwell 96 Plate.
2. Add 400 µL of sample to each sample well.
3. Add 400 µL of Nuclease-free Water (not DEPC-Treated) to the Negative Control well.
4. Invert the Binding Bead Mix 5 times gently to mix, then add 550 µL to each sample well and Negative Control well.

   Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The Binding Bead Mix is viscous, so pipet slowly to ensure that the correct amount is added. DO NOT reuse pipette tips to add Binding Bead Mix to the samples, as the high viscosity will cause variations in the volumes added.

5. Add 10 µL of MS2 Phage Control to each sample well and to the Negative Control well.
6. Seal the plate with MicroAmp™ Clear Adhesive Film, then shake the sealed plate at 1,050 rpm for 2 minutes.
7. Incubate the sealed plate at 65°C for 5 minutes (ensure the bottom of the plate is uncovered), then shake the plate at 1,050 rpm for 5 minutes.
8. Place the sealed plate on the magnetic stand for 10 minutes or until all of the beads have collected.

Wash the beads (400-µL sample input volume)

1. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

2. Remove the plate from the magnetic stand, then add 1 mL of Wash Buffer to each sample.
3. Reseal the plate, then shake at 1,050 rpm for 1 minute.
4. Place the plate back on the magnetic stand for 2 minutes, or until all the beads have collected.
5. Keeping the plate on the magnet, carefully remove the cover, then discard the supernatant from each well.

   IMPORTANT! Avoid disturbing the beads.

6. Repeat step 2 to step 5 using 1 mL of 80% Ethanol.
7. Repeat step 2 to step 5 using 500 µL of 80% Ethanol.
8. Dry the beads by shaking the plate (uncovered) at 1,050 rpm for 2 minutes.

Elute the nucleic acid (400-µL sample input volume)

1. Add 50 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.
2. Shake the sealed plate at 1,050 rpm for 5 minutes.
3. Place the plate in an incubator at 65°C for 10 minutes.
4. Remove the plate from the incubator, then shake the plate at 1,050 rpm for 5 minutes.
5. Place the sealed plate on the magnetic stand for 3 minutes or until clear to collect the beads against the magnets.
6. Keeping the plate on the magnet, carefully remove the seal, transfer the eluates to a fresh standard (not deep-well) 96-well plate, then seal the plate with MicroAmp™ Clear Adhesive Film.

   IMPORTANT! To prevent evaporation, seal the plate containing the eluate immediately after the transfers are complete.

Note: Significant bead carry over may adversely impact RT-PCR performance.

Place the plate on ice for immediate use in real-time RT-PCR.