MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit

Manual isolation of viral and pathogen nucleic acid (RNA and DNA) from biofluids and transport media

Catalog Number A42356

Pub. No. MAN0018074 **Rev.** B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Applied Biosystems™ MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit is developed for scalable, rapid purification of high-quality nucleic acid (RNA and DNA) from virus, bacteria, and yeast/fungi in biofluid and transport media samples. You can use the nucleic acid purified with this kit in a broad range of molecular biology downstream applications, such as sequencing and real-time PCR. This protocol guides users through manual isolations in plate format using a magnetic stand.

Contents and storage

Reagents that are provided in the kit are sufficient for 100 reactions.

Table 1 Components of MagMAX™ Viral/Pathogen Ultra Nucleic Acid Isolation Kit (Cat. No. A42356)

Component	Amount	Storage
Binding Solution	53 mL	15°C to 25°C
Wash Buffer	100 mL	
Elution Solution	10 mL	
Proteinase K	1 mL	
Total Nucleic Acid Binding Beads	2 mL	
Enzyme Mix	5 mL	–15°C to 25°C

For 1,000 reaction volume, use Cat. No. A42359 (Binding Solution), A42360 (Wash Buffer), A42364 (Elution Solution), A42363 (Proteinase K), A42362 (Binding Beads), and A42366 (Enzyme Mix).

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Equipment	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Vortex	MLS
Magnetic Stand-96	AM10027
Compact Digital Microplate Shaker	88880023
Incubator capable of reaching 65°C with slatted shelves	MLS
Consumables	
Deep-well plates:	
KingFisher™ Deepwell 96 Plate	95040450
KingFisher™ 96 KF microplate	97002540
Materials	
MicroAmp™ Clear Adhesive Film	4306311
Conical Tubes (15 mL)	AM12500
Conical Tubes (50 mL)	AM12501
Reagent reservoirs	MLS
Nonstick, RNase-Free Microfuge Tubes, 1.5 mL	AM12450
Nonstick, RNase-Free Microfuge Tubes, 2.0 mL	AM12475
Reagents	
Ethanol, 100% (molecular biology grade)	MLS
Nuclease-free Water	AM9932

General guidelines

- Perform all steps at room temperature (20–25°C), unless otherwise noted.
- Precipitates can occur if the Binding Solution is stored when room temperature is too cold. If there are precipitates, warm the Binding Solution at 37°C and gently mix to dissolve the precipitates. Avoid creating bubbles.

- Reagent Mix tables are sufficient for a single reaction. To calculate volumes for other sample numbers, see the per-well volume and add at least 10% overage.
- If using a plate shaker other than the recommended shaker, ensure that:
  - The plate fits securely on the plate shaker.
  - The recommended speeds are compatible with the plate shaker. Ideal shaker speeds allow for thorough mixing without splashing.

Guidelines for Binding Bead Mix

- Vortex Binding Beads thoroughly before each use.
- Ensure that the beads stay fully mixed within the solution during pipetting.
- Avoid creating bubbles during mixing and aliquoting.
- Binding/Bead Mix is very viscous so pipet with care to ensure that the correct volume is added to the sample.

Before first use of the kit

- Prepare 80% Ethanol from 100% absolute Ethanol and Nuclease-Free Water.
  - Prepare enough for 1.5 mL per reaction.

(Optional) Before each use of the kit with standard volume of vaginal microbiota and urinary tract samples

1. Add 1 mL of sample per well of a 96 deep-well plate.
2. Cover the plate, then centrifuge for 15 minutes at 2,250 × *g*.
3. Remove as much of the supernatant as possible, then add 200 µL of 1X PBS to each sample.
4. Proceed to "Perform total nucleic acid purification using 200–400 µL" on page 2.

Prepare Binding Bead Mix

1. Vortex Beads vigorously to ensure they are homogenous.
2. Prepare Binding Bead Mix according to the following table and sample input volume:

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

[1] Use 10% Overage calculation when making a master mix for use with multiple samples.

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

Perform total nucleic acid purification using 200–400 µL

1 Digest with Enzyme Mix

a. Gently swirl the Enzyme Mix, then add 50 µL per sample well in a Deep-well 96-well plate.

This is the Sample Plate.

b. Add 200–400 µL of sample to wells with Enzyme Mix, then seal the plate with MicroAmp™ Clear Adhesive Film.

Note: Recommend up to 200 µL input for whole blood.

c. Shake the sealed plate at 1,050 rpm for 5 minutes, then incubate for 15 minutes at 65°C (ensure the bottom of the plate is uncovered).

d. Shake the sealed plate at 1,050 rpm for 5 minutes.

2 Digest with Proteinase K

a. Remove the plate from the incubator, then add 10 µL of Proteinase K to each sample.

b. Invert the Binding Bead Mix gently to mix, then add 550 µL to each sample.

Note: Remix the Binding Bead Mix by inversion frequently during pipetting to ensure even distribution of beads to all samples or wells. The mixture containing the Binding Beads is viscous. Therefore, pipet slowly to ensure that the correct amount is added. DO NOT use a repeat pipet to add to the samples as the high viscosity will cause variations in volume added.

c. Seal the plate, then shake the sealed plate at 1,050 rpm for 2 minutes.

d. 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.

e. Place the sealed plate on the magnetic stand for 10 minutes, or until all of the beads have collected.

3 Wash the beads

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

IMPORTANT! Avoid disturbing the beads.

b. Remove the plate from the magnetic stand, then add 1 mL of Wash Buffer to each sample.

c. Reseal the plate, then shake at 1,050 rpm for 1 minute.

d. Place the plate back on the magnetic stand for 2 minutes, or until all the beads have collected.

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

IMPORTANT! Avoid disturbing the beads.

f. Repeat step 3b to step 3e using 1 mL of 80% Ethanol.

g. Repeat step 3b to step 3e using 500 µL of 80% Ethanol.

h. Dry the beads by shaking the plate (uncovered) at 1,050 rpm for 2 minutes.

4 Elute the nucleic acid

a. Add 50–100 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.

b. Shake the sealed plate at 1,050 rpm for 5 minutes.

c. Place the plate in an incubator at 65°C for 10 minutes.

d. Remove the plate from the incubator, then shake the plate at 1,050 rpm for 5 minutes.

e. Place the sealed plate on the magnetic stand for 3 minutes or until clear to collect the beads against the magnets.

f. Keeping the plate on the magnet, carefully remove the seal, then transfer the eluates to a fresh standard (not deep-well) plate.

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

The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long-term storage.

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Revision	Date	Description
B.0	06 December 2019	Updated Total Nucleic Acid Binding Buffer to Binding Solution.
A.0	15 March 2019	New document.

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