MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit for HIV-1 Dried Blood Spots

For manual extraction of HIV-1 viral RNA from dried blood spots (DBS) and HIV-1 positive plasma in plate format using a magnetic stand

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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 Nucleic Acid Isolation Kit for HIV-1 Dried Blood Spots is designed to recover viral RNA from dried blood spots (DBS) for HIV-1 drug resistance testing. An alternative protocol is also provided for processing of plasma samples. The kit utilizes MagMAX™ magnetic-bead technology, to ensure reproducible recovery of high-quality HIV-1 viral RNA compatible with downstream applications such as RT-PCR and Sanger sequencing.

Contents and storage

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

Component	Amount	Storage
Binding Solution	55 mL	15°C to 25°C
MagMAX™ Dried Blood Spots Lysis Solution	60 mL	
Wash Buffer	100 mL	
Elution Solution	10 mL	
Proteinase K	1 mL	
Total Nucleic Acid Binding Beads	2 mL	

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
Equipment	
Adjustable and multi-channel micropipettors	MLS
Vortex mixer	MLS
Incubator with slatted shelves capable of reaching 65°C	MLS
Magnetic Stand-96	AM10027
Iris scissors, stainless steel or Deaver scissors, stainless steel	50-109-3849 50-109-3335
Compact Digital Microplate Shaker or equivalent [1]	88880023
Twist shaker (Daigger FINEPCR TW3T, HulaMixer™ Sample Mixer, or equivalent)	MLS
Materials and consumables	
KingFisher™ 96 Deep-Well Plate	95040450
KingFisher™ 96 KF microplate	97002540
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
5 mg/mL Linear polycrylamide (LPA)	AM9520
Recommended Downstream Assays	
HIV-1 Genotyping Kit: Amplification Module	A32317

[1] If using a plate shaker other than the recommended shaker, ensure that the plate fits securely on the plate shaker and that recommended speeds are compatible with the plate shaker. Ideal shaker speeds allow for thorough mixing without splashing.

General guidelines

- Perform all steps at room temperature (20–25°C), unless otherwise noted.
- Volumes for reagent mixes are given for a single well. It is recommended to prepare a master mix for larger sample numbers. To calculate volumes for master mixes, multiply the per-well volume appropriately and add 10–15% overage to account for differences in pipetting.
- If precipitate is observed in the Binding Solution, warm the solution at 37°C and gently mix to dissolve the precipitates. Avoid creating bubbles.
- Binding Solution and Binding Bead Mix are very viscous. Pipet the solutions slowly and with care, ensuring tips are pre-wet prior to dispensing. Change tips frequently if necessary.

Guidelines for cutting dried blood spots

- Use clean stainless steel scissors to cut out a circular spot of ~13mm diameter (or slightly larger).
- Cut as close to the perimeter of the spot without actually touching the spotted blood.
- Use a new, or clean pair of scissors for each new donor specimen.
- Clean scissors with 10% household bleach followed by 70% ethanol (the 70% ethanol is important to prevent corrosion of the metal).
  After cleaning, ensure scissors are dry before storage.

  Note: To avoid any potential unwanted chemical reactions, do not mix bleach waste with liquid waste containing DBS Lysis Solution or Binding Solution.

Before first use of the kit

IMPORTANT! Wash Solution may develop inert white or brown particulates that float in solution. This is not a cause for concern and does not negatively affect performance.

- Prepare 80% ethanol from 100% absolute ethanol and nuclease-free water.

  Prepare enough for 1.5 mL per reaction.

Perform viral RNA extraction from dried blood spots

1 Perform dried blood spot lysis

1.1. Place a ~13mm diameter circular dried blood spot sample (containing 100 µL dried blood) into an appropriately sized tube (screw cap test tube, or 1.5-mL microcentrifuge tube).

1.2. Add 600 µL of MagMAX™ Dried Blood Spots Lysis Solution to the tube.

1.3. Add 10 µL of Proteinase K to the tube.

1.4. Lay the tube horizontally on a rocker (TW3T twist shaker with non-slip pad to prevent rolling) and incubate the sample at room temperature for 30 minutes with gentle rocking (a setting of 9 or greater on a TW3T twist shaker).

Note: During this time, the color from the blood spot should disappear from the substrate and the solution should turn brown.

1.5. Centrifuge the tube to collect the solution at bottom of tube.

2 Prepare Binding Bead Mix

2.1. Vortex Beads vigorously to ensure they are homogenous.

2.2. 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.

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

3 Add Binding Bead Mix

3.1. Transfer 350–400 µL of each DBS lysate sample to each well of a Deep-well 96-well plate.

This plate is the Sample Plate.

3.2. Invert Binding Bead Mix gently to mix, then add 550 µL to each sample in the Sample Plate.

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.

3.3. Seal the plate with MicroAmp™ Clear Adhesive Film, then shake the sealed plate at 1,050 rpm for 2 minutes.

3.4. 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.

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

4 Wash the beads

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

IMPORTANT! Avoid disturbing the beads.

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

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

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

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

IMPORTANT! Avoid disturbing the beads.

4.6. Repeat step 4.2 to step 4.5 using 1 mL of 80% Ethanol.

4.7. Repeat step 4.2 to step 4.5 using 500 µL of 80% Ethanol.

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

5 Elute the nucleic acid

5.1. Add 30 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.

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

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

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

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

5.6. Keeping the plate on the magnet, carefully remove the seal, then transfer the eluates to a standard (not deep-well) 96-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.

Perform viral RNA extraction from plasma (200 µL)

1 Prepare Binding Bead Mix

1.1. Vortex Beads vigorously to ensure they are homogenous.

1.2. Prepare Binding Bead Mix according to the following table and sample input volume:

Component	Volume per well [1]	Volume per well [1] (For low copy samples [2])
Binding Solution	530 µL	530 µL
Total Nucleic Acid Magnetic Beads	20 µL	20 µL
5 mg/mL linear polyacrylamide (LPA) [2]	—	1 µL
Total volume	**550 µL**	**551 µL**

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

Note: If samples containing low to mid-level viral loads (<5,000 copies/mL) result in poor yields, adding LPA as a carrier to enhance recovery of nucleic acid is recommended.

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

2 Digest with Proteinase K

2.1. Add 10 µL of Proteinase K to each well of a Deep-well 96-well plate.

This plate is the Sample Plate.

2.2. Add 200 µL of each plasma sample to wells with Proteinase K in the Sample Plate.

2.3. Invert Binding Bead Mix gently to mix, then add 550 µL (551 µL if using LPA) to each sample in the Sample Plate.

Note: Invert the tube containing the Binding Bead Mix frequently during pipetting to ensure even distribution of beads to wells. Do not vortex (to avoid bubbles).

The Binding Bead Mix is viscous and requires slow pipetting to ensure the correct volume is added. **Do not** use a repeater pipette to add samples because the high viscosity will cause variation in the volumes dispensed.

2.4. Seal the plate with MicroAmp™ Clear Adhesive Film, then shake the sealed plate at 1,050 rpm for 2 minutes.

2.5. 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.

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

3 Wash the beads

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

IMPORTANT! Avoid disturbing the beads.

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

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

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

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

IMPORTANT! Avoid disturbing the beads.

3 Wash the beads *(continued)*

3.6. Repeat step 3.2 to step 3.5 using 1 mL of 80% Ethanol.

3.7. Repeat step 3.2 to step 3.5 using 500 µL of 80% Ethanol.

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

4 Elute the nucleic acid

4.1. Add 30 µL of Elution Solution to each sample, then seal the plate with MicroAmp™ Clear Adhesive Film.

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

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

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

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

4.6. Keeping the plate on the magnet, carefully remove the seal, then transfer the eluates to a standard (not deep-well) 96-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.

Limited product warranty

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Revision	Date	Description
A.0	5 April 2023	New manual for MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.