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

For extraction of HIV-1 viral RNA from dried blood spots (DBS) and HIV-1 positive plasma using the KingFisher™ Duo Prime instrument

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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	
KingFisher™ Duo Prime Magnetic Particle Processor	5400110
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Magnetic Stand-96	AM10027
Iris scissors, stainless steel or Deaver scissors, stainless steel	50-109-3849 50-109-3335
Twist shaker (Daigger FINEPCR TW3T, HulaMixer™ Sample Mixer, or equivalent)	MLS
Materials and consumables	
KingFisher™ 96 Deep-Well Plate	A48305 95040450
KingFisher™ 12-tip comb for Microtiter 96 Deepwell plate	97003500
KingFisher™ Elution Strip	97003520
KingFisher™ Duo Cap for Elution Strip	97003540
MicroAmp™ Clear Adhesive Film	4306311
Conical Tubes (15 mL)	AM12500
Conical Tubes (50 mL)	AM12501
Reagent reservoirs	MLS
Reagents	
Ethanol, 100% (molecular biology grade)	MLS
5 mg/mL Linear polycrylamide (LPA)	AM9520
Recommended Downstream Assays	
HIV-1 Genotyping Kit: Amplification Module	A32317

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.

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.

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 starting

- Set up the KingFisher™ Duo Prime instrument with the 12-tip magnetic head and a 12 well heat strip.
- Load the KingFisher™ Duo Prime instrument with the **MVP_Duo** instrument script (see the *Thermo Scientific™ KingFisher™ Duo User Manual* for details on downloading the script).

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 Prepare Sample Plate and Elution Strip

Prepare the Sample Plate and Elution Strip according to the following tables.

Table 1 Sample Plate

Plate row	Row ID	Prefill
A	Sample	Sample
B	—	Empty
C	Wash 1	1000 µL Wash Buffer
D	—	Empty
E	Wash 2	1000 µL 80% ethanol
F	—	Empty
G	Wash 3	500 µL 80% ethanol
H	Tip comb plate	Tip comb

Table 2 Elution Strip

Plate row	Row ID	Prefill
A	Elution	30 µL Elution Solution

4 Run automated protocol

4.1. Transfer 350–450 µL of each DBS supernatant into the sample wells (Row A) of the Sample Plate.

Note: Do not exceed 450 µL to avoid spill-over from the plate.

4.2. Add 550 µL of Binding Bead Mix to each sample well.

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.

4.3. Load the script called **MVP_Duo** on the instrument, and push **Start** to initiate the protocol.

4.4. Load the Elution Strip and Sample Plate onto instrument as directed on the instrument screen.

4.5. Immediately after the run (~27 minutes) remove the Elution Strip from the instrument.

4.6. Cover the Elution Strip with an Elution Strip Cap for temporary storage, or transfer the samples to a tube or plate for final storage.

4.7. Store the nucleic acid samples on ice for immediate use in downstream assay, or 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 Prepare Sample Plate and Elution Strip

Prepare the Sample Plate and Elution Strip according to the following tables.

Table 3 Sample Plate

Plate row	Row ID	Prefill
A	Sample	Sample
B	—	Empty
C	Wash 1	1000 µL Wash Buffer
D	—	Empty
E	Wash 2	1000 µL 80% ethanol
F	—	Empty
G	Wash 3	500 µL 80% ethanol
H	Tip comb plate	Tip comb

Table 4 Elution Strip

Plate row	Row ID	Prefill
A	Elution	30 µL Elution Solution

3 Digest with Proteinase K

3.1. Add 10 µL of Proteinase K to each sample well (Row A) of the Sample Plate.

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

3.3. 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.

4	Run automated protocol	**4.1.** Load the script called **MVP_Duo** on the instrument, and push **Start** to initiate the protocol. **4.2.** Load the Elution Strip and Sample Plate onto instrument as directed on the instrument screen. **4.3.** Immediately after the run (~27 minutes) remove the Elution Strip from the instrument. **4.4.** Cover the Elution Strip with an Elution Strip Cap for temporary storage, or transfer the samples to a tube or plate for final storage. **4.5.** Store the nucleic acid samples on ice for immediate use in downstream assay, or at –20°C for long term storage.

Limited product warranty

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

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Revision	Date	Description
A.0	3 February 2022	New document for MagMAX Viral Pathogen Total Nucleic Acid Isolation DBS (KingFisher Duo Prime instrument protocol).