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HighPrep™ Viral RNA Kit - KingFisher™ Format

OPTIMIZED PROTOCOL FOR SARS-CoV-2 RNA ISOLATION

Manual Revision v1.0
Catalog No. KFV-R96, KFV-R2000

- Isolation of viral nucleic acids from viral transport media (VTM), plasma, swabs, saliva, whole blood, and other bodily fluids.
- Magnetic beads based chemistry

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For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

Product Description

The HighPrep™ Viral RNA Kit - KingFisher™ Format is designed for rapid and reliable isolation of viral nucleic acids from various viral transport media, whole blood, serum, plasma, swabs, saliva, and other bodily fluids. The kit extracts high quality viral RNA that is suitable for direct use in most downstream applications such as amplification and enzymatic reactions. It can be adapted to most major liquid handling workstations in the market.

Process

Samples are lysed in a specially formulated buffer containing detergent. Nucleic acid is bound to the surface of MAG-S1 particles under proper condition. Proteins and cellular debris are efficiently washed with few wash steps. Pure RNA is then eluted in Elution Solution. Purified RNA can be directly used in downstream applications without the need for further purification.

Kit Contents and Storage

HighPrep™ Viral RNA Kit - KingFisher™ Alternative Catalog No.	**KFV-R96 (Sample)**	**KFV-R2000**	**STORAGE**
Number of Preps	96	2000	
Binding Solution	22 ml	460 ml	15-25°C
Wash Buffer[1]	30 ml	650 ml	15-25°C
Elution Solution	8 ml	120 ml	15-25°C
Pro K Solution[2]	1.1 ml	22 ml	2-8°C
MAG-S1 Particles	1.1 ml	22 ml	2-8°C
LES I[3]	5 ml	110 ml	2-8°C

[1]Ethanol must be added prior to use. See Preparation of Reagents Section.

Stability

All components are stable for 14 months when stored accordingly.

[2] Pro K Solution comes in a ready to use solution. Pro K is stable for 12 months when stored at 15-25°C. For storage longer than 1 year, store at 2-8°C.

[3]LES I Buffer comes in a ready to use solution and is stable at 2-8°C (30 days). For longer storage, keep at -20°C.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Resource" tab when viewing the product kit.

Viral RNA - 200 µl sample volume (96 well plate format/single tube)
OPTIMIZED PROTOCOL FOR SARS-Cov-2 RNA ISOLATION

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ Ethanol (80%)
- ☐ Isopropanol (100%)
- ☐ Magnetic separation device for 96 well plate/1.5ml - 2ml (MagBio Catalog No. MYMAG-96 and MYMAG-96X).
- ☐ 96 well microplates (U or V bottom) or 1.5-2ml microcentrifuge tubes

Things to do before starting

Preparation of Reagents:

Prepare the following components for each kit before use:

Wash Buffer

Catalog No.	Component	Add 100% Ethanol	Storage
KFV-R96 (Sample)	Wash Buffer	20 ml	Room Temp 15-25°C
Components are stable for 14 months when stored accordingly.			

Catalog No.	Component	Add 100% Ethanol	Storage
KFV-R2000	Wash Buffer	400 ml	Room Temp 15-25°C
Components are stable for 14 months when stored accordingly.			

Binding Bead Mix

Vortex Beads vigorously to ensure they are homogenous.

Prepare Binding Bead Mix according to the following table:

Component	Volume per reaction	Volume for 96 preps (Sample)	Volume for 2000 preps
Binding Solution	212 µl	22 ml	460 ml
MAG-S1 Particles	10 µl	1.1 ml	22 ml
100% Isopropanol	58 µl	6 ml	120 ml
Total Volume	**280 µl**	**29.1 ml**	**602 ml**

Volume per well: 280 µl

NOTE: The order of plate preparation is reagents first and the sample plate last.

Preparation of Wash and Elution Plates (200-µL sample input volume)

Prepare the processing plates as indicated below. The order of plate preparation is reagents first and the sample plate last. In order to avoid alcohol evaporation and contamination, seal the filled plate with MicroAmp™ Clear Adhesive Film and then move on to the next plate.

Plate ID	Plate Position	Plate Type	Reagent	Volume Per Well
Wash 1 Plate	2	Deep-Well 96 Plate	Wash Buffer	500 µL
Wash 2 Plate	3		80% Ethanol	500 µL
Elution Plate	4		Elution Buffer	50 µL
Tip Comb Plate	5	Place a 96-tip comb for DW magnets in a Deep-Well 96 Plate.		

Sample Pretreatment Step: 10 mins reaction time

1. **Gently swirl LES I container, then pipette 50 µl to each well/tube.**
2. **Add 200 µl of sample to each well/tube. Pipette mix 15 times.**

   ⚠ Note: If sample is less than 200 µl, bring volume up to 200 µl with Elution Solution.
3. **Incubate for 10 mins at 37°C.**

The following are the brief steps to perform in reference to the MVP_2Wash_200_Flex protocol:

1. **Add 10 µl of Pro K Solution to 250 ul of pretreated sample mix (in the tube or well).**
2. **Invert Binding Bead Mix gently to mix, then add 280 µl to each sample. This plate is the "sample plate."**
3. **Select the program MVP_2Wash_200_Flex on the instrument.**
4. **Start the run, then load the prepared plates into position when prompted by the instrument.**
5. **After the protocol is complete (~25 minutes after start), immediately remove the elution plate from the instrument and cover the plate or transfer the eluate to a tube or plate of choice for final storage.**
6. **The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –80°C for long term storage.**

Troubleshooting guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:
Phone: 301-302-0144 (in US), outside US, 1-855-262-4246
Email: support@magbiogenomics.com

Symptoms	Possible Causes	Comments
Low RNA Yield	Incomplete resuspension of MAG-S1 Particles.	Resuspend MAG-S1 Particles by vortexing vigorously before use.
	Loss of MAG-S1 Particles during operation.	Avoid disturbing the MAG-S1 Particles during aspiration of supernatant.
	Ethanol is not added into Wash Buffer.	Add absolute 100% Ethanol to Wash Buffer (see page 2 for instructions).
	Inefficient cell lysis.	Double the volume of Pro K Solution and incubate longer.
MAG-S1 Particles do not completely clear from solution	Too short of magnetizing time.	Increase collection time on the magnet. Make sure the solution is completely clear before discarding the supernatant.
Problems in downstream applications	Insufficient RNA in starting material	Use more starting material.
	Ethanol carry-over.	Dry the MAG-S1 Particles completely before elution. Use a fine pipette tip to pipette out any residual liquid during the drying of beads.
Carryover of MAG-S1 Particles	The eluate has particles and is not fully clear.	Increase magnetization time. If small amount of carryover, place eluted sample on a magnetic separation device and perform an additional 5 min magnetization.

Ordering Information

Product Description	Catalog No.	Preps
HighPrep™ Viral RNA Kit (KingFisher™ Format) 2,000 preps	KFV-R2000	2,000

Related Products

Next Gen library prep clean-up system

Product Description	Catalog No.
HIghPrep™ RNA Elite (5 mL)	RC-90005
HIghPrep™ RNA Elite (50 mL)	RC-90050
HIghPrep™ RNA Elite (250 mL)	RC-90250
HIghPrep™ RNA Elite (500 mL)	RC-90500

Magnetic Separation Devices

Product Description	Catalog No.
Handheld Magnetic Separation Device (96 well microplate format)	MYMAG-96
Magnetic Separation Device (96 well ring magnet plate)	MYMAG-96X
MagStrip magnetic stand (1.5 mL x 12)	MBMS-12
15ml and 50ml magnetic stand combo. (3x15ml and 3x50ml)	MBMS-31550


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