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MagSep Viral DNA/RNA Kit

Instructions for use

003 0452.909-02/012015

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1 Operating instructions

1.1 Using this manual

- Before using this product for the first time, please read the entire manual.
- Read the software manual and the hardware manual of the epMotion.
- This manual is an intrinsic part of the product and should be kept in an accessible location.

1.2 Danger symbols and danger levels

The safety instructions of this operating manual indicate the following danger symbols and danger levels:

1.2.1 Danger symbols

 **General danger**	

1.2.2 Danger levels

DANGER	*Will* lead to severe injuries or death.
WARNING	*May* lead to severe injuries or death.
CAUTION	May lead to light to moderate injuries.
NOTICE	May lead to material damage.

1.3 Symbols used

Depiction	Meaning
1. 2.	Actions in the specified order
▸	Actions without a specified order
•	List
Text	Display text or software text
	Additional information

2 Product description

2.1 Main illustration

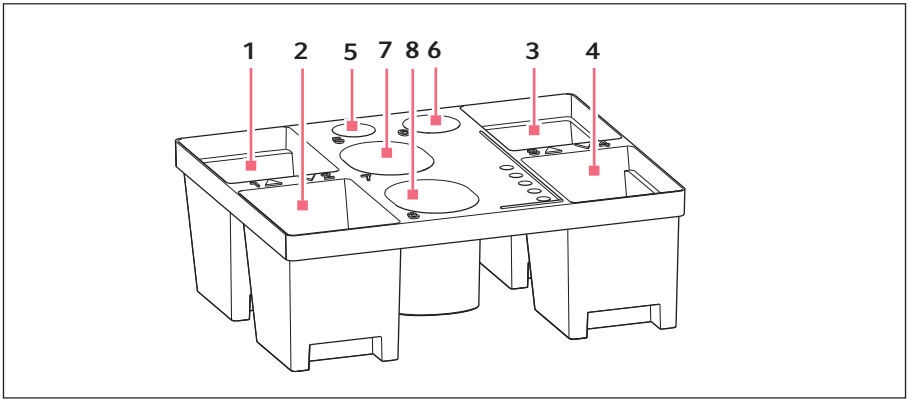


Fig. 2-1: MagSep Viral DNA/RNA Kit

1 Viral BB (Binding Buffer)
2 Viral WB 1 (Wash Buffer)
3 Viral WB 2 (Wash Buffer)
4 Viral WB 3 (Wash Buffer)
5 Viral Beads
6 Viral ProK (Proteinase K)
7 Viral LB (Lysis Buffer)
8 Viral EB (Elution Buffer)

The numbering of the picture corresponds to the number of reagents in the MagSep Viral DNA/RNA Kit.

2.2 Delivery package

Quantity	Description	Portion
4	Viral BB (Binding Buffer)	18 mL
4	Viral WB 1 (Wash Buffer)	15 mL
4	Viral WB 2 (Wash Buffer)	15 mL
4	Viral WB 3 (Wash Buffer)	16 mL
4	Viral Beads	0.9 mL
4	Viral ProK (Proteinase K)	10 mg
4	Viral LB (Lysis Buffer)	6 mL
4	Viral EB (Elution Buffer)	3 mL
4	Viral PB (Proteinase Buffer)	0.8 mL
4	Carrier RNA	90 µg
4	Safe-Lock tubes 2.0 mL DNA LoBind, Pack of 50 tubes	
1	Manual for MagSep Viral DNA/RNA Kit	

3 Safety

3.1 Intended use

MagSep Viral DNA/RNA Kit components were developed, designed, distributed, and sold solely for research purposes. They are only suitable for in-vitro applications. No claim or representation is intended for its use for identifying any specific organism, or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

Rather, it is the responsibility of the user to verify the use of the MagSep Viral DNA/RNA Kit for a specific application range, because the performance characteristic of this kit has not been verified for a specific organism.

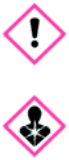
3.2 Warnings for intended use

DANGER! Danger due to reagents in the MagSep Viral DNA/RNA Kit.
MagSep Kits contain hazardous contents.

- Wear your personal protective equipment.
- Follow the safety instructions in the Safety signs chapter.

3.3 Safety signs on the reagents

Component	Hazard content	GHS symbol			Hazard phrases	Precaution phrases	CAS
Viral LB (Lysis Buffer)	Guanidinium chloride 50% – 66%			WARNING			50-01-1
Viral BB (Binding Buffer)	Ethanol 35% – 55% Sodium perchlorate 20% – 40%	 	GHS02 GHS07	WARNING			64-17-5 7601-8 9-0
Viral WB 1 (Wash Buffer)	Ethanol 20% – 35%		GHS02	WARNING			64-17-5
Viral WB 2 (Wash Buffer)	Ethanol 55% – 75%		GHS02	DANGER			64-17-5
Viral WB 3 (Wash Buffer)	Ethanol 80%		GHS02	DANGER			64-17-5

Component	Hazard content	GHS symbol			Hazard phrases	Precaution phrases	CAS
Viral ProK (Proteinase K)	Proteinase K		GHS07 GHS08	DANGER	H315 H317 H319 H334 H335	P261 P264 P271 P272 P280 P302+P352 P304+P340 P305+P351 +P338 P312 P333+P313 P337+P313 P342+P311 P363 P403+P233 P405	39450-01-6

3.3.1 Hazard phrases

H315	Causes skin irritation.
H317	May cause an allergic skin reaction.
H319	Causes serious eye irritation.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335	May cause respiratory irritation.

3.3.2 Precaution phrases

P261	Avoid breathing dust/fumes/gas/mist/vapors/spray.
P264	Wash thoroughly after handling.
P271	Use only outdoors or a well-ventilated area.
P272	Contaminated work clothing shoud not be allowed out of the workplace.
P280	Wear protective gloves/eye protection.
P302+352	IF ON SKIN: Wash with plenty of soap and water.
P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305+P351+ P338	IF IN EYES: Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do - continue rinsing.
P312	Call a POISON CENTER of doctor/physician if you feel unwell.
P333+P313	If skin irritation or a rash occurs: Get medical advice/attention.
P337+P313	If eye irritation persists get medical advice/attention.
P342+P311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor/ physician.
P363	Wash contaminated clothing before reuse.
P403+P233	Store in a well ventilated place. Keep container tightly closed.
P405	Store locked up.

4 Operation

4.1 The basic principle

The MagSep Viral DNA/RNA Kit is designed for the isolation of viral DNA or RNA from cell-free body fluids such as serum or plasma. This MagSep Viral DNA/RNA Kit provides reagents and magnetic beads for isolation of 1 - 24 samples from 200 µL of cell-free biological fluids. The procedure is based on the reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions. Sample lysis is achieved via incubation in a solution containing chaotropic ions. DNA viruses (e.g., HBV) are usually more difficult to lyse and require additional Proteinase K digestion. Viral BB and the Viral Beads are added to the lysate to bind nucleic acids to the paramagnetic beads. Carrier RNA improves binding and recovery of low-concentrated viral RNA. After magnetic separation and removal of the supernatant, the paramagnetic beads are washed three times to remove contaminants and salt, followed by a drying step at high temperatures.

Finally, highly pure viral DNA/RNA is eluted with low-salt Viral EB. Purified viral DNA/RNA can be directly used for downstream applications.

The MagSep Viral DNA/RNA Kit is optimized for use with the Eppendorf epMotion automated pipetting system.

4.1.1 Kit specifications

The MagSep Viral DNA/RNA Kit is designed for rapid, automated, small-scale preparation of highly pure viral DNA/RNA from 200 µL cell-free body fluids (e.g., serum, plasma, urine) using the 3D MagSep technology on the epMotion system. The obtained DNA/RNA can be immediately used as a template for RT-PCR, PCR, or any kind of enzymatic reaction. The actual processing time depends on the number of samples per batch.

Carrier RNA (poly(-A) RNA: poly(A) potassium salt, prepared from ADP with polynucleotide phosphorylase) is included for optimal performance.

Carrier RNA enhances binding of viral nucleic acids to the magnetic beads and reduces the risk of viral RNA degradation. Please note that eluates contain both viral nucleic acids and Carrier RNA and the amount of Carrier RNA may exceed the amount of viral nucleic acids. Therefore, when using the Carrier RNA, the isolated nucleic acids cannot be quantified with the kit via photometric or fluorometric methods. Thus, other methods for quantification, such as specific quantitative PCR or RT-PCR systems, are recommended. Carrier RNA may inhibit PCR. Therefore, the amount of added Carrier RNA may be carefully optimized according to the PCR system used.

4.2 Preparations

4.2.1 Sample preparation

4.2.1.1 Liquid sample

Biological fluids or semi-fluid samples can be processed, e.g. serum, urine or bronchoalveolar lavage. For successful nucleic acid purification, it is important to obtain a homogeneous, clear, and non-viscous sample.

1. Check all samples for presence of precipitates, especially old or frozen samples.
2. Avoid clearing samples before lysis, because viruses of interest may be associated with particles or aggregates.

4.2.1.2 Solid sample (tissue sample, stool sample)

1. Prepare a 10 % (w/v) suspension of tissue in buffer (e.g., PBS) using commercial homogenization tools like rotor-stator or bead-based homogenization tools.
2. Centrifuge the suspension in order to remove particles.
3. Use the clear, particle-free supernatant for further processing.

4.2.1.3 Swab material

1. Incubate the swab in a suitable buffer (e.g., PBS) or cell-culture medium for 30 min.
2. Proceed with the particle-free buffer or medium.

4.2.2 Preparing the components of the MagSep Viral DNA/RNA Kit

All buffers are delivered ready-to-use.

4.2.2.1 Handling of Viral Beads

A homogeneous distribution of the magnetic beads to each tube in the PrepRack is essential for high tube-to-tube consistency.

1. Before loading the tray onto the epMotion, make sure that the Viral Beads are completely resuspended.
2. Thoroughly shake the storage vial, or briefly place it on a vortexer.
3. To avoid an incorrect level measurement result from the optical sensor, make sure that there are no drops of Viral Beads in the cap or on the internal vial wall.
4. Place the vial correctly in the tray. Carefully push down the vial after reinserting it into the tray.

4.2.2.2 Preparing Viral ProK (Proteinase K) solution

We recommend preparing a fresh Proteinase K solution for each set of 24 reagents.

The addition of Viral ProK (Proteinase K) solution is necessary for the isolation of viral DNA or simultaneous viral DNA/RNA isolation. For isolation of viral RNA, Viral ProK (Proteinase K), treatment is not usually required. Viral ProK (Proteinase K) treatment is

recommended for viral RNA isolation when viscous samples have to be processed, e.g., sputum samples.

1. Add 0.6 mL of Viral PB (Proteinase Buffer) and mix gently to dissolve lyophilized Viral ProK (Proteinase K).
   This Proteinase K solution is stable at -20 °C for up to 6 months.
2. Before next use mix gently the Proteinase K solution.

4.2.2.3 Carrier RNA

For the isolation of viral RNA from fluid biological samples, add Carrier RNA to Viral LB (Lysis Buffer)

1. Add 1 mL Viral LB (Lysis Buffer) to the Carrier RNA vial.
2. Dissolve the RNA and transfer it back to the Viral LB (Lysis Buffer) bottle.
   Viral LB (Lysis Buffer), including Carrier RNA, can be stored at room temperature for 1 – 2 weeks.
   Viral LB (Lysis Buffer), including Carrier RNA, can be stored at 4 °C for up to 4 weeks. Storage at 4 °C may cause salt precipitation.
3. Place Viral ProK (Proteinase K) in Position 6.
4. If the solution was stored at 4 °C , it must be preheated at 40 °C for a maximum of 5 min in order to redissolve salts.

Do not warm Viral LB (Lysis Buffer) containing Carrier RNA more than 4 times!

Frequent warming, temperatures > 80 °C, and extended heat incubation will accelerate the degradation of Carrier RNA. This leads to reduced recovery of viral RNA, and eventually false negative RT-PCR results, particularly if low titer samples are used.

4.2.3 Starting process

Prerequisites

- epMotion is ready for operation.
- Make sure that all samples and all kit components are prepared as described.

1. Transfer 200 µL of the sample material in 2 mL tubes provided with the kit.
2. Place the sample/elution tubes row-by-row in the racks, starting at position 1.
3. For viral DNA and viral RNA isolation, including the ProK digest, place Viral ProK (Proteinase K) solution at position 6 in the tray.
4. Vortex the Viral Beads.
5. Open the reagent bottles.
6. Empty the liquid waste.
7. Start the application Prep Assistant *MagSep Viral DNA/RNA* at the EasyCon.
   The software assistant guides you through the set-up for automated nucleic acid purification.

4.3 Process

Procedure in the epMotion for

- MagSep Viral RNA
- MagSep Viral RNA including ProK digest
- MagSep Viral DNA

Steps in procedure	Command	Description
Lyse sample	Reagent Transfer	Dispense 10 µL Proteinase K (only MagSep Viral RNA including ProK digest and MagSep Viral DNA).
	Thermomixer	Shake for 30 s, 1000 rpm (only MagSep Viral RNA including ProK digest and MagSep Viral DNA).
	Reagent Transfer	Dispense 200 µL Viral LB (Lysis Buffer).
	Thermomixer	Shake for 10 min, 1200 rpm, 25 °C (only MagSep Viral DNA 56 °C).
Bind NA to Viral Beads	Reagent Transfer	Dispense 600 µL Viral BB (Binding Buffer).
	Thermomixer	Shake for 30 s, 1200 rpm.
	Reagent Transfer	Dispense 30 µL Viral Beads.
	Thermomixer	Shake for 5 min, 1000 rpm, 25 °C.
	Separation	Separate for 2 min.
	Pool one Destination	Remove supernatant.
	Thermomixer	Shake for 30 s, 1200 rpm.
1st wash	Reagent Transfer	Dispense 500 µLViral WB 1 (Wash Buffer).
	Thermomixer	Shake for 2 min, 1200 rpm, 25 °C.
	Separation	Separate for 2 min.
	Pool one Destination	Remove supernatant.
	Thermomixer	Shake for 30 s, 1200 rpm.
2nd wash	Reagent Transfer	Dispense 500 µL Viral WB 2 (Wash Buffer).
	Thermomixer	Shake for 2 min, 1200 rpm, 25 °C.
	Separation	Separate for 2 min.
	Pool one Destination	Remove supernatant.
	Thermomixer	Shake for 30 s, 1200 rpm.

Steps in procedure	Command	Description
3rd wash	Reagent Transfer	Dispense 550 µL Viral WB 3 (Wash Buffer).
	Thermomixer	Shake for 2 min, 1200 rpm, 25 °C.
	Separation	Separate for 2 min.
	Pool one Destination	Remove supernatant.
Drying step	Thermomixer	Incubate for 7 min, 55 °C.
	Thermomixer	Shake for 7 min, 1200 rpm, 55 °C.
Elution	Reagent Transfer	Dispense 25 – 100 µL Viral EB (Elution Buffer).
	Thermomixer	Shake for 5 min, 1200 rpm, 25 °C (only MagSep Viral DNA 56 °C).
	Separation	Separate for 2 min.
	Sample Transfer	Transfer eluted DNA.

4.4 Finishing

4.4.1 Completing the process

- Close the reagent bottles tightly after use. Alcohol may evaporate.

4.4.2 Elution procedures

Elution of purified genomic DNA can be carried out at a volume of 25 µL – 100 µL. For an optimal ratio of yield and concentration, perform the elution depending on your sample material with a volume of 50 µL.

For optimal performance of isolated DNA/RNA in subsequent downstream applications, we recommend storage, especially long term, at -20 °C.

5 Troubleshooting

Problem	Cause	Solution
Salt ingredients of Viral LB (Lysis Buffer) or Viral BB (Binding Buffer) precipitate.	• Storage at low temperatures.	▶ Incubate the buffer at 40 °C and shake well.
Poor or no DNA yield.	• Wrong bottle arrangement in the tray.	▶ Check the position of all reagents in the tray.
	• Salt ingredients of Viral LB (Lysis Buffer) or Viral BB (Binding Buffer) precipitate.	▶ Incubate the buffer at 40 °C and shake well.
Carry-over of beads	• Not suitable or too much sample material.	▶ Decrease the used sample material.
Suboptimal performance of DNA in downstream applications.	• Wrong array in the tray.	▶ Check the position of all reagents in the tray.
	• Salt ingredients of Viral LB (Lysis Buffer) or Viral BB (Binding Buffer) precipitate.	▶ Incubate the buffer at 40 °C and shake well.
	• Ethanol evaporation from wash buffers.	▶ Close reagent bottles tightly after use.
Cross contamination.	• The *re-use tips* option is selected even though a SafeRack is not loaded.	▶ Be sure that a SafeRack is loaded. ▶ Be sure that the option *re-use tips* is not selected.
Faulty level detection.	• Vial is not correctly positioned.	▶ Make sure that the vial is placed on the bottom of the tray. Carefully press the vial down after reinserting it into the tray.
	• Bead solution on the internal vial wall.	▶ Make sure that there are no drops of bead solution in the cap or on the internal vial wall.

6 Transport, storage and disposal

6.1 Storage conditions

Reagent bottles must be stored tightly closed. Alcohol may evaporate.

Buffers and Beats of the MagSep Viral DNA/RNA Kit	Stable at 18 °C – 25 °C for up to one year. Do not store below 18 °C, as salt ingredients may precipitate.
Viral ProK (Proteinase K) solution	Stable at -20 °C for up to 6 months.
Viral LB (Lysis Buffer) with Carrier RNA	Stable at 4 °C for up to 4 weeks. Storage at 4 °C may cause salt precipitation. Preheat the buffer at 40 °C for a maximum of 5 min in order to redissolve salts.

7 Technical data

Technology	Magnetic bead technology
Sample material	200 µL cell-free body fluids
Elution volume	25 µL – 100 µL

8 Ordering information

8.1 Recommended pipette tips

epT.I.P.S. Motion SafeRack are intended for the reuse of tips within an epMotion application. They feature compartments which separates adjacent tips. The compartments prevent cross contamination of residual liquid in used tips. The use of epT.I.P.S. Motion SafeRacks is recommended when the *Re-use tips* option is selected in the software assistant.

Order no. (International)	Description
	epT.I.P.S. Motion Filter 50 µL 10 SafeRacks with 96 tips each
0030 014.618	PCR clean
	epT.I.P.S. Motion Filter 1 000 µL 10 SafeRacks with 96 tips each
0030 014.650	PCR clean

8.2 Alternative pipette tips

Order no. (International)	Description
	epT.I.P.S. Motion 50 µL 10 racks with 96 tips each
0030 014.405	Eppendorf Quality
0030 015.207	Sterile
	epT.I.P.S. Motion 1 000 µL 10 racks with 96 tips each
0030 014.480	Eppendorf Quality
0030 015.240	Sterile
	epT.I.P.S. Motion Filter 50 µL 10 racks with 96 tips each
0030 014.413	PCR clean
0030 015.215	PCR clean and Sterile
	epT.I.P.S. Motion Filter 1 000 µL 10 racks with 96 tips each
0030 014.499	PCR clean
0030 015.258	PCR clean and Sterile
	epT.I.P.S. Motion 50 µL 10 SafeRacks with 96 tips each
0030 014.600	Eppendorf Quality
	epT.I.P.S. Motion 1 000 µL 10 SafeRacks with 96 tips each
0030 014.642	Eppendorf Quality

8.3 MagSep Kits

Order no. (International)	Description
	MagSep Tissue gDNA Kit
0030 450.000	Reagent kit for DNA purification of 4 × 24 tissue and cell samples
	MagSep Blood gDNA Kit
0030 451.007	Reagent kit for DNA purification of 4 × 24 blood samples
	MagSep Viral DNA/RNA Kit
0030 452.003	Reagent kit for viral DNA/RNA purification of 4 × 24 cell free biological fluid samples.

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