MAGBIO

"WE MAKE NGS BETTER"

HighPrep™ Viral/Bacterial DNA-RNA Kit

Manual Revision v1.0
Catalog Nos. HVB-DR96, HVB-DR96X4

- Isolation of viral and bacterial total nucleic acids from different types of viral transport media, plasma, swabs, saliva, urine, whole blood, and other bodily fluids.
- Magnetic beads based chemistry

Contents

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/Bacterial DNA-RNA Kit is designed for rapid and reliable isolation of total nucleic acids from viruses and bacteria in whole blood, serum, plasma, swabs, saliva, urine, and other bodily fluids. The kit extracts high quality DNA and 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 special conditions. Proteins and cellular debris are efficiently washed with few wash steps. Pure RNA and DNA are then eluted in Nuclease-Free Water or low ionic strength buffer. Purified RNA or DNA can be directly used in downstream applications without the need for further purification.

Kit Contents and Storage

HighPrep™ Viral/Bacterial DNA-RNA Kit Catalog No.	**HVB-DR96**	**HVB-DR96X4**	**STORAGE**
Number of Preps*	96	384	
Viral Lysis Buffer	30 ml	120 ml	15-25°C
RDW Buffer[1]	30 ml	120 ml	15-25°C
Nuclease-Free Water	35 ml	140 ml	15-25°C
Pro K Solution[2]	2.2 ml	8.8 ml	2-8°C
NBE[3]	2 ml	8 ml	2-8°C
MAG-S1 Particles	1.1 ml	4.4 ml	2-8°C
LES I[3]	5 ml	20 ml	2-8°C

[1]Ethanol must be added prior to use. See Preparation of Reagents Section.
*The number of preps indicated on the table above are based on a 200 µl sample preparation protocol.

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]NBE and LES I Buffers come in a ready to use solution and are 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.

Preparation of Reagents

Prepare the following components for each kit before use:

Catalog No.	Component	Add 100% Ethanol	Storage
HVB-DR96	RDW Buffer	20 ml	Room Temp 15-25°C
Components are stable for 14 months when stored accordingly.			

Catalog No.	Component	Add 100% Ethanol	Storage
HVB-DR96X4	RDW Buffer	80 ml	Room Temp 15-25°C
Components are stable for 14 months when stored accordingly.			

Number of Reactions

Kit Catalog No.	Number of preparations based on sample volume*	
	200 µl	**400 µl**
HVB-DR96	110 preps	55 preps
HVB-DR96X4	440 preps	220 preps

*The number of preparations indicates how many reactions can be performed out of each kit based on the volume of sample processed.

A - VIRAL NUCLEIC ACID ISOLATION PROTOCOLS

1. Viral DNA/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 magnetic separation device
- ☐ 96 well microplates (U or V bottom) or 1.5 - 2ml microcentrifuge tubes

Things to do before starting

☐ Prepare all reagents accordingly according to the instructions on page 2.

Protocol

1. **Gently swirl LES I container, then pipette 50µl 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 Nuclease-Free Water.

3. **Incubate for 10 mins at 37°C.**

4. **Add 240 µl of Viral Lysis Buffer and 10µl of Pro K Solution. Mix very well.**

5. **Incubate at 56°C - 60°C for 5 min. May use a thermoshaker. If there is none in the lab, make sure to shake the samples once or twice during incubation.**

6. **Let the samples cool to room temperature and add 8 µl of NBE, 280 µl of Isopropanol, and 10 µl of MAG-S1 Particles. Pipette mix 15 times.**

   ⚠ Shake well to resuspend the MAG-S1 Particles before use.

7. **Let the samples sit at room temperature for 5 min.**

8. **Place the sample plate on the magnetic separation device for 3 min to magnetize the MAG-S1 Particles or until the magnetic beads clear from solution.**

9. **With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.**

10. **Remove the plate from the magnetic separation device and add 400 µl of RDW Buffer. Pipette mix 15 times to re-suspend the MAG-S1 Particles until solution is homogeneous. Then place sample back on magnetic rack and wait for 3 mins to magnetize particles. Remove the supernatant.**

11. **Remove the plate/tube from magnetic rack. Then add 500 µl of 80% ethanol and mix to re-suspend magnetic bead particles.**

    ⚠ Note: Make sure the solution is homogeneous.

12. **Place sample back on the magnetic rack and wait for 3 mins to magnetize particles or until the magnetic beads clear from solution.**

13. **Discard supernatant and then repeat steps 11 - 12 for a 2nd wash.**

14. **Discard the supernatant and air-dry the beads for 7 mins.**

15. **Remove the plate from the magnetic separation device. Add 30-100 µl of Nuclease-Free Water to each well/tube and pipette mix 15 times to completely re-suspend the MAG-S1 Particles.**

    ⚠ Complete resuspension of the MAG-S1 Particles is crucial for better yield.

16. **Incubate at 56-60°C for 5 min.**

17. **Place the sample plate back on the magnetic separation device and wait for 3 min or until the magnetic beads clear from solution.**

18. **Transfer the eluate (cleared supernatant containing DNA or RNA) to a new micro-plate for storage. Store DNA at -20°C and RNA at -80°C.**

2. Viral DNA/RNA - 400 µl sample volume (96 well plate format/single tube)

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 magnetic separation device
- ☐ 96 well microplates (U or V bottom) or 1.5 - 2ml microcentrifuge tubes

Things to do before starting

- ☐ Prepare all reagents accordingly according to the instructions on page 2.

Protocol

1. **Gently swirl LES I container, then pipette 100 µl to each well/tube.**

2. **Add 400 µl of sample to each well/tube. Pipette mix 15 times.**

   ⚠ Note: If sample is less than 400 µl, bring volume up to 400 µl with Nuclease-Free Water.

3. **Incubate for 10 mins at 37°C.**

4. **Add 400 µl of Viral Lysis Buffer and 20 µl of Pro K Solution. Mix very well.**

5. **Incubate at 56°C - 60°C for 5 min. May use a thermoshaker. If there is none in the lab, make sure to shake the samples once or twice during incubation.**

6. **Let the samples cool to room temperature and add 12 µl of NBE, 400 µl of Isopropanol, and 20 µl of MAG-S1 Particles. Pipette mix 15 times.**

   ⚠ Shake well to resuspend the MAG-S1 Particles before use.

7. **Let the samples sit at room temperature for 5 min.**

8. **Place the sample plate on the magnetic separation device for 3 min to magnetize the MAG-S1 Particles or until the magnetic beads clear from solution.**

9. **With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.**

10. **Remove the plate from the magnetic separation device and add 400 µl of RDW Buffer. Pipette mix 15 times to re-suspend the MAG-S1 particles until solution is homogeneous. Then place sample back on magnetic rack and wait for 3 mins to magnetize particles. Remove the supernatant.**

11. Remove the plate/tube from magnetic rack. Then add 400 µl of 80% ethanol and mix to re-suspend magnetic bead particles.

⚠ Note: Make sure the solution is homogeneous.

12. Place sample back on the magnetic rack and wait for 3 mins to magnetize particles or until the magnetic beads clear from solution.

13. Discard supernatant and then repeat steps 11 - 12 for a 2nd wash.

14. Discard the supernatant and air-dry the beads for 7 mins.

15. Remove the plate from the magnetic separation device. Add 30-100 µl of Nuclease-Free Water to each well/tube and pipette mix 15 times to completely re-suspend the MAG-S1 Particles.

⚠ Complete resuspension of the MAG-S1 Particles is crucial for better yield.

16. Incubate at 56-60°C for 5 min.

17. Place the sample plate back on the magnetic separation device and wait for 3 min or until the magnetic beads clear from solution.

18. Transfer the eluate (cleared supernatant containing DNA or RNA) to a new micro-plate for storage. Store DNA at -20°C and RNA at -80°C

B - BACTERIAL NUCLEIC ACID ISOLATION PROTOCOLS

1. Bacterial DNA/RNA in Urine - 200 µl sample volume (96 well plate format/single tube)

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 magnetic separation device
- ☐ 96 well microplates (U or V bottom) or 1.5 - 2ml microcentrifuge tubes

Things to do before starting

- ☐ Prepare all reagents accordingly according to the instructions on page 2.

Protocol

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 Nuclease-Free Water.

3. **Incubate for 10 mins at 37°C.**

4. **Add 240 µl of Viral Lysis Buffer and 20 µl of Pro K Solution. Mix very well.**

5. **Incubate at 60°C - 65°C for 10 min. May use a thermoshaker. If there is none in the lab, make sure to shake the samples once or twice during incubation.**

6. **Let the samples cool to room temperature and add 8 µl of NBE, 280 µl of Isopropanol, and 10 µl of MAG-S1 Particles. Pipette mix 15 times.**

   ⚠ Shake well to resuspend the MAG-S1 Particles before use.

7. **Let the samples sit at room temperature for 5 min.**

8. **Place the sample plate on the magnetic separation device for 3 min to magnetize the MAG- S1 particles or until the magnetic beads clear from solution.**

9. **With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.**

10. **Remove the plate from the magnetic separation device and add 400 µl of RDW Buffer. Pipette mix 15 times to re-suspend the MAG-S1 Particles until solution is homogeneous. Then place sample back on magnetic rack and wait for 3 mins to magnetize particles. Remove the supernatant.**.

11. Remove the plate/tube from magnetic rack. Then add 500 µl of 80% ethanol and mix to re-suspend magnetic bead particles.

⚠ Note: Make sure the solution is homogeneous.

12. Place sample back on the magnetic rack and wait for 3 mins to magnetize particles or until the magnetic beads clear from solution.

13. Discard supernatant and then repeat steps 11 - 12 for a 2nd wash.

14. Discard the supernatant and air-dry the beads for 7 mins.

15. Remove the plate from the magnetic separation device. Add 30-100 µl of Nuclease-Free Water to each well/tube and pipette mix 15 times to completely re-suspend the MAG-S1 Particles.

⚠ Complete resuspension of the MAG-S1 Particles is crucial for better yield.

16. Incubate at 56-60°C for 5 min.

17. Place the sample plate back on the magnetic separation device and wait for 3 min or until the magnetic beads clear from solution.

18. Transfer the eluate (cleared supernatant containing DNA or RNA) to a new micro-plate for storage. Store DNA at -20°C and RNA at -80°C.

2. Bacterial DNA/RNA in Urine - 400 µl sample volume (96 well plate format/single tube)

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 magnetic separation device
- ☐ 96 well microplates (U or V bottom) or 1.5 - 2ml microcentrifuge tubes

Things to do before starting

- ☐ Prepare all reagents accordingly according to the instructions on page 2.

Protocol

1. **Gently swirl LES I container, then pipette 100 µl to each well/tube.**

2. **Add 400 µl of sample to each well/tube. Pipette mix 15 times.**

   ⚠ Note: If sample is less than 400 µl, bring volume up to 400 µl with Nuclease-Free Water.

3. **Incubate for 10 mins at 37°C.**

4. **Add 500 µl of Viral Lysis Buffer and 20 µl of Pro K Solution. Mix very well.**

5. **Incubate at 60°C - 65°C for 10 min. May use a thermoshaker. If there is none in the lab, make sure to shake the samples once or twice during incubation.**

6. **Let the samples cool to room temperature and add 12 µl of NBE, 500 µl of Isopropanol, and 10 µl of MAG-S1 Particles. Pipette mix 15 times.**

   ⚠ Shake well to resuspend the MAG-S1 Particles before use.

7. **Let the samples sit at room temperature for 5 min.**

8. **Place the sample plate on the magnetic separation device for 3 min to magnetize the MAG- S1 particles or until the magnetic beads clear from solution.**

9. **With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.**

10. **Remove the plate from the magnetic separation device and add 400 µl of RDW Buffer. Pipette mix 15 times to re-suspend the MAG-S1 particles until solution is homogeneous. Then place sample back on magnetic rack and wait for 3 mins to magnetize particles. Remove the supernatant.**.

11. **Remove the plate/tube from magnetic rack. Then add 500 µl of 80% ethanol and mix to re-suspend magnetic bead particles.**

    ⚠ Note: Make sure the solution is homogeneous.

12. **Place sample back on the magnetic rack and wait for 3 mins to magnetize particles or until the magnetic beads clear from solution.**

13. **Discard supernatant and then repeat steps 11 - 12 for a 2nd wash.**

14. **Discard the supernatant and air-dry the beads for 7 mins.**

15. **Remove the plate from the magnetic separation device. Add 30-100 µl of Nuclease-Free Water to each well/tube and pipette mix 15 times to completely re-suspend the MAG-S1 Particles.**

    ⚠ Note: Complete resuspension of the MAG-S1 Particles is crucial for better yield.

16. **Incubate at 56-60°C for 5 min.**

17. **Place the sample plate back on the magnetic separation device and wait for 3 min or until the magnetic beads clear from solution.**

18. **Transfer the eluate (cleared supernatant containing DNA or RNA) to a new micro-plate for storage. Store DNA at -20°C and RNA at -80°C.**

3. Bacterial DNA/RNA in Whole Blood - 200 µl sample volume (96 well plate format/ single tube)

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 magnetic separation device
- ☐ 96 well microplates (U or V bottom) or 1.5 - 2ml microcentrifuge tubes

Things to do before starting

- ☐ Prepare all reagents accordingly according to the instructions on page 2.

Protocol

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 Nuclease-Free Water.

3. **Incubate for 10 mins at 37°C.**

4. **Add 240 µl of Viral Lysis Buffer and 20 µl of Pro K Solution. Mix very well.**

5. **Incubate at 60°C - 65°C for 10 min. May use a thermoshaker. If there is none in the lab, make sure to shake the samples once or twice during incubation.**

6. **Let the samples cool to room temperature and add 8 µl of NBE, 280 µl of Isopropanol, and 10 µl of MAG-S1 Particles. Pipette mix 15 times.**

   ⚠ Shake well to resuspend the MAG-S1 Particles before use.

7. **Let the samples sit at room temperature for 5 min.**

8. **Place the sample plate on the magnetic separation device for 3 min to magnetize the MAG-S1 Particles or until the magnetic beads clear from solution.**

9. **With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.**

10. **Remove the plate from the magnetic separation device and add 400 µl of RDW Buffer. Pipette mix 15 times to re-suspend the MAG-S1 particles until solution is homogeneous. Then place sample back on magnetic rack and wait for 3 mins to magnetize particles. Remove the supernatant.**.

11. **Remove the plate/tube from magnetic rack. Then add 500 µl of 80% ethanol and mix to re-suspend magnetic bead particles.**

    ⚠ Note: Make sure the solution is homogeneous.

12. **Place sample back on the magnetic rack and wait for 3 mins to magnetize particles or until the magnetic beads clear from solution.**

13. **Discard supernatant and then repeat steps 11 - 12 for a 2nd wash.**

14. **Discard the supernatant and air-dry the beads for 7 mins.**

15. **Remove the plate from the magnetic separation device. Add 30-100 µl of Nuclease-Free Water to each well/tube and pipette mix 15 times to completely re-suspend the MAG-S1 Particles.**

    ⚠ Note: Complete resuspension of the MAG-S1 Particles is crucial for better yield.

16. **Incubate at 56-60°C for 5 min.**

17. **Place the sample plate back on the magnetic separation device and wait for 3 min or until the magnetic beads clear from solution.**

18. **Transfer the eluate (cleared supernatant containing DNA or RNA) to a new micro-plate for storage. Store DNA at -20°C and RNA at -80°C.**

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 DNA or 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 RDW Buffer.	Add absolute 100% Ethanol to RDW 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 DNA/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 separtion device and perform an additional 5 min magnetization.

Ordering Information

Product Description	Catalog No.	Preps
HighPrep™ Viral/Bacterial DNA-RNA (96 preps)	HVB-DR96	96
HighPrep™ Viral/Bacterial DNA-RNA (384 preps)	HVB-DR96X4	384

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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