USER GUIDE

# *MagListo*™ 5M Viral DNA/RNA Extraction Kit

Cat. No. K-3623
K-3624

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# *MagListo*™ 5M Viral DNA/RNA Extraction Kit

**Kit for the extraction of DNA/RNA from virus**

# User Guide

**K-3623** Σ **500**

**K-3624** Σ **100**

**Version No.: 1 (2022-05-25)**

**Please read all the information in booklet before using the unit**


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## Intended Use

*MagListo*™ 5M Viral DNA/RNA Extraction Kit is developed and supplied for research purposes only. Certain applications possible with this kit may require special approval by appropriate local and/or national regulatory authorities in the country of use.

## Safety Warning and Precaution

Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. For more information, please consult the appropriate Material Safety Data Sheet (MSDS).

## Warranty and Liability

All BIONEER products undergo extensive Quality Control testing and validation. BIONEER guarantees quality during the warranty period as specified, when following the appropriate protocol as supplied with the product. It is the responsibility of the purchaser to determine the suitability of the product for its particular use. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days.

## Quality Management System ISO 9001 Certified

Every aspect of our quality management system from product development, production to quality assurance and supplier qualification meets the world-class standards.

## Patent

*MagListo*™ and its kits are protected by the patents KR10-2015-0089172.

## Trademark

*MagListo*™ is a trademark of BIONEER Corporation.

## Copyright



## Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

# Contents

## Product Information

### Components

| Components | K-3624 (100 rxn) | K-3623 (500 rxn) | Storage |
|---|---|---|---|
| Proteinase K powder, lyophilized | 25 mg x 1 ea | 25 mg x 5 ea | Refer to the “Storage” below. |
| Poly(A), lyophilized | 2 mg x 1 ea | 5 mg x 2 ea | |
| Magnetic Nano Bead | 22 ml x 1 ea | 110 ml x 1 ea | Store at room temperature (15-25°C). |
| VB Buffer (Binding) | 50 ml x 1 ea | 250 ml x 1 ea | |
| VWM1 Buffer (1st Washing) | 50 ml x 2 ea | 125 ml x 2 ea | |
| RWA2 Buffer (2nd Washing) | 100 ml x 1 ea | 250 ml x 2 ea | |
| WE Buffer (3rd Washing) | 120 ml x 1 ea | 300 ml x 2 ea | |
| ER Buffer (Elution) | 20 ml x 1 ea | 100 ml x 1 ea | |
| One Page Protocol | 1 ea | 1 ea | |

### Storage

The kit will maintain performance for at least two years under standard storage conditions.

All components of the kit should be stored dry at room temperature (15-25°C). Before use, lyophilized Proteinase K and Poly(A) should be completely dissolved in 1,250 µl and 500 µl of nuclease-free water, respectively. For short term storage, dissolved Proteinase K and Poly(A) should be stored at 4°C. For long term storage, it is recommended to aliquot these enzymes into separate tubes and store at -20°C.

*** Note:** Please note that repeated freezing and thawing may reduce its activity.

## Specifications

| ***MagListo*™ 5M Viral DNA/RNA Extraction Kit** | |
|---|---|
| Sample Type | Serum, plasma, CSF, saliva |
| Amount of Starting Sample | up to 200 µl |
| Turnaround Time | < 30 min |
| Elution Volume | 50-100 µl |
| Isolation Technology | Magnetic Nano Bead |

*** Note:** There may be differences in measured values depending on the type of samples.

## Precautions

- Take appropriate laboratory safety precautions and wear gloves when handling because VB Buffer and VWM1 Buffer contain chaotropic salts which are irritants.
- RNA is highly susceptible to degradation by exogenous RNase that may be introduced during the handling steps, all the steps must be conducted under sterile, RNase-free condition.
- RNase-free reagents, pipette tips, and tubes must be used with gloved hands while handling them.

# Introduction

## Product Description

*MagListo*™ 5M Viral DNA/RNA Extraction Kit is designed for extraction of highly purified DNA or RNA from serum, plasma, or other cell-free body fluids. The kit employs Magnetic Nano Beads to extract viral DNA/RNA with the aid of *MagListo*™ Magnetic Separation Rack and *ExiPrep*™ 96 Lite (Cat. No. A-5250). The use of *MagListo*™ Magnetic Separation Rack along with the kit greatly increases user convenience by shortening the extraction time without centrifugation. On the same principle, *ExiPrep*™ 96 Lite is designed for rapid extraction of nucleic acids delivering up to 96 extracted samples (including controls).

DNA/RNA extracted through this kit can be used for a variety of applications, including: reverse transcription PCR (RT-PCR), reverse transcription quantitative PCR (RT-qPCR), northern blot analysis, pathogen detection and cDNA synthesis. We recommend DNase treatment for only RNA quantitation.

## Principle

*MagListo*™ 5M Viral DNA/RNA Extraction Kit uses Magnetic Nano Beads to extract nucleic acid. Buffers within the kit assist nucleic acid to bind to silica-coated magnetic nanobeads. As a result, high yield and highly purified nucleic acid is extracted from samples.

The kit consists of lysis & binding buffer, washing buffer, elution buffer, and magnetic nanobeads. Samples are lysed and homogenized in the presence of a guanidine-thiocyanate-containing buffer, which is a highly denaturing agent and inactivates RNase to isolate RNA. Extracted DNA/RNA is bound to silica-coated magnetic nanobeads. Proteins and other contaminants are eliminated by subsequent washing, and highly purified DNA or RNA is eluted in an elution buffer.

*** Note:** Some samples require pre-treatment step.

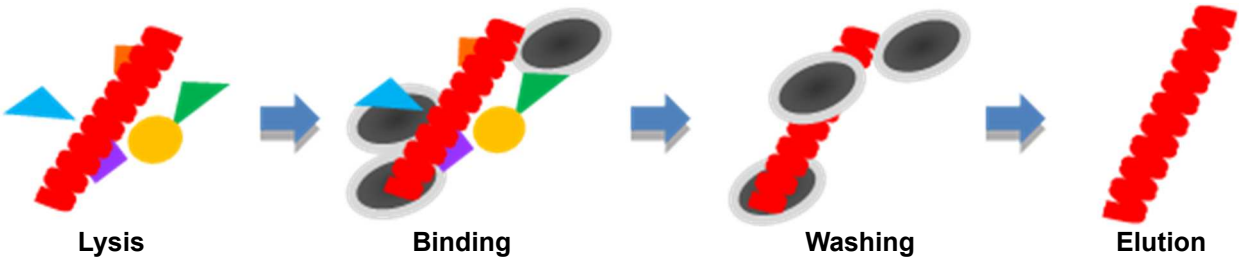



**Figure 1. Nucleic acid extraction using silica-coated magnetic nanobeads.**

## Features & Benefits

- Comprehensive: High quality and yield of DNA/RNA extraction from various samples such as serum, plasma, CSF, or saliva.
- Rapid: Magnetic Nano Beads enable the rapid nucleic acid extraction.
- Ready-to-use: Extracted DNA/RNA is ready-to-use for various application.
- Minimized DNA/RNA damage: DNA/RNA damage is minimized by avoiding precipitation and use of organic solvents.
- Cost-effective: Can be applied to *ExiPrep*™ 96 Lite to automate DNA/RNA extraction.

## Magnetic Nano Beads

Magnetic Nano Beads have been developed to overcome defects of existing resin and to automate purification process. The principle of extraction using the magnetic nanobeads is that coated functional group on the surface of it binds to nucleic acid. Magnetic nanobeads are then isolated using external magnetic field.

### Specification

| Silica-coated Magnetic Nano Beads | |
|---|---|
| Matrix | Silica-coated $Fe_3O_4$ |
| Average size | 400 nm |
| Ligand | - OH |
| Working Temperature | 0-100°C |
| Storage | Store at room temperature. |

### Features & Benefits

- Rapid: Fast binding guarantees high throughput automation.
- Effectiveness: Large surface area enables more sensitive assay.
- Specificity: Globular structure increases specificity by decreasing non-specific binding.

## *MagListo*™ Magnetic Separation Rack

*MagListo*™ Magnetic Separation Rack is designed for a fast and easy separation of the Magnetic Nano Beads. BIONEER offers various racks of different sizes - *MagListo*™-8Ch for 8-tube strip and muti-pipette, *MagListo*™-2 for 2 ml tube, *MagListo*™-15 for 15 ml tube, and *MagListo*™-50 for 50 ml tube. These racks consisting of different size allow user to choose the product according to their needs.

**The followings are recommended when handling the *MagListo*™ Magnetic Separation Rack.**

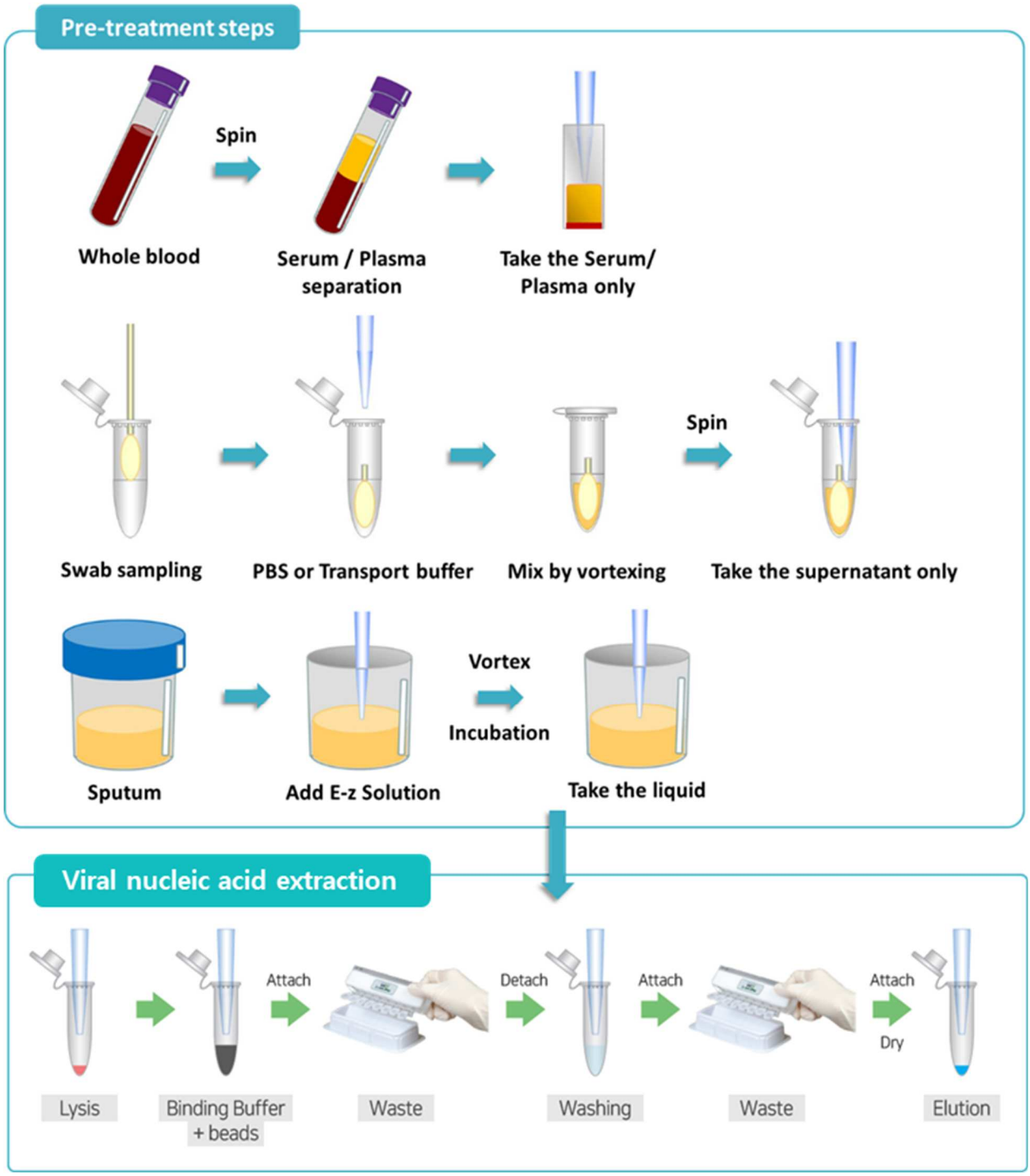
- The product is made of acryl and plastic. Be careful not to drop the product as the dropping may break the product.
- When moving the product, take extra care not to drop the product as it may cause injury.
- If the product is broken, do not discard it with bare hands as the sharp edges may cause injury.
- When an extracted or purified nucleic acid is spilled on the product, immediately rinse it with running water and clean it with 70% ethanol.
- Acetone, toluene, or organic solvent may cause damage to the acrylic and plastic part of the product, which may lead to malfunction of the product. Rinse the product immediately when the above-mentioned solvent leaks as the expected DNA yield may not be obtained if the product is damaged.
- Check the magnet plate part of the product for corrosive liquid. In the event of a sill, it may corrode the magnet during storage and may deteriorate performance, so rinse immediately with running water.

**Features & Benefits**

- Rapid: Isolates plasmid DNA, genomic DNA, and/or RNA quickly (within 20 minutes) and economically.
- Convenient: Simply disposes waste solution just by flipping the rack without centrifugation or pipetting as the silicon fixture secures the tube.

## Experimental Procedures

### Procedure Overview



## Sample Preparation

Several factors such as harvesting method and storage of starting samples can influence the yield and RNA purity. All specimens must be stored at -70°C or used immediately after collection. It is recommended to put the sample as soon as possible on ice and avoid repeated freezing and thawing. Repeated freezing and thawing of the sample will result in RNA degradation.

### Plasma

Plasma should be collected in a tube containing anticoagulants for blood (EDTA and ACDs). Sample can be stored for several days at 4°C and for up to 1 year at -70°C. It is recommended to defrost the sample rapidly in a water bath (37°C) and store it on ice before use.

### Serum

Serum should be collected in a tube containing a serum separator (SST Tube). Sample can be stored for 7 days at 4°C and for up to 1 year at -70°C. It is recommended to defrost the sample rapidly in a water bath (37°C) and store it on ice before use.

### Swab

Insert the swab through one nostril straight back (not upwards), along the bottom of the nasal passage until reaching the nasopharynx. Rotate it several times to obtain secretions. Remove the swab and apply it into the specimen collection tube. Break the swab shaft and leave the swab in the tube. Samples to be tested can be stored for 1 day at room temperature, for 4 days at 2-8°C, and for a long time at -20°C.

### Sputum

Collect sputum specimen by inducing a cough into a sterile container. Sample to be tested can be stored for 1 day at room temperature, for 4 days at 2-8°C, and for a long time at -20°C.

## Before You Begin

Before proceeding, please check the following:

1. Completely dissolve Proteinase K powder in 1,250 µl of nuclease-free water before use. Dissolved Proteinase K should be stored at 4°C.

2. Dissolve Poly(A) in 500 µl of nuclease-free water and mix gently by vortexing. Add dissolved Poly(A) solution to VB Buffer and mix thoroughly.
   * **Note:** In this case, Poly(A) can be degraded by RNase under the external environment. So, it is recommended to use and store it on a clean bench.

3. Add indicated volume of absolute ethanol (not provided) to VWM1 Buffer before use (see bottle label).

4. Pre-heat ER Buffer at 60°C before use. This process is essential for maximal recovery in filter-based extraction.

## Viral DNA/RNA Extraction

1. Apply 200 µl of serum, plasma, urine, or CSF sample to a 1.5 ml or 2 ml tube (not provided).
   * **Note:** For swab sample, add PBS buffer (not provided) to the sample and mix by vortexing. Use only supernatant.

2. Add 10 µl of Proteinase K to the sample from step 1.

3. **(Lysis)** Add 200 µl of VB Buffer† and mix briefly by vortexing for 10 seconds.
   * **Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.
   † Refer to the No.2 of “Before You Begin” on page 10.

4. Incubate at 56-60°C for 10 minutes.

5. **(DNA/ RNA precipitation)** Add 400 µl of absolute ethanol (not provided) and mix briefly by vortexing for 10 seconds. Briefly spin down to collect lysate clinging under the lid.

6. **(DNA/ RNA binding)** Add 200 µl of Magnetic Nano Bead solution to each tube and mix thoroughly by vortexing until the beads are fully resuspended.

7. Place the tube in *MagListo*™-2 Magnetic Separation Rack with the magnet plate attached and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

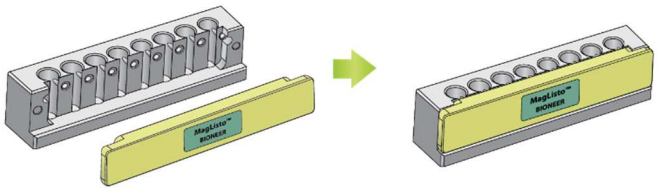


**Figure 2. Attachment of the magnet plate.** Combine the magnet plate to the stand.

8. Without removing the tube from *MagListo*™-2 Magnetic Separation Rack, discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting. During this process, the beads containing nucleic acid remain attached to the side of the tube.

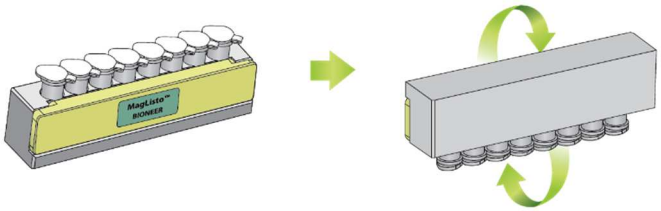


**Figure 3. How to discard the supernatant.** Discard the supernatant by inverting the *MagListo*™-2 Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

9. **(1st Washing)** Detach the magnet plate from *MagListo*™-2 Magnetic Separation Rack. Add 700 µl of VWM1 Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended.

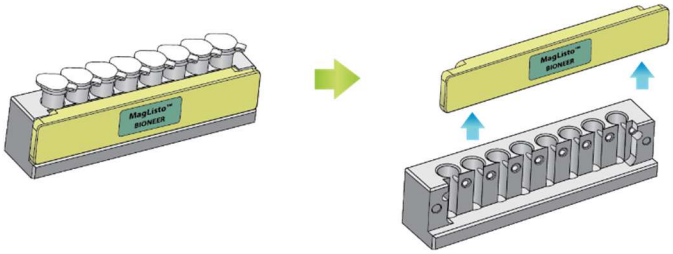


**Figure 4. Detachment of the magnet plate.** Pull the magnet plate up and gently separate it.

10. Attach the magnet plate to *MagListo*™-2 stand and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

11. Without removing the tubes from *MagListo*™-2 Magnetic Separation Rack, discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.

12. Repeat steps 9-11.

13. **(2nd Washing)** Detach the magnet plate from *MagListo*™-2 Magnetic Separation Rack. Repeat steps 9-11 by adding 700 µl of RWA2 Buffer for additional washing. Repeat steps 10-11 once more.

14. **(3rd Washing)** Remove residual ethanol according to step 14-A or 14-B.

14-A. Washing beads:

Without removing the tube from *MagListo*™-2 Magnetic Separation Rack, add 700 µl of WE Buffer to the opposite side of beads. Close the cap and invert the rack twice in order to remove ethanol from the sample. Discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.

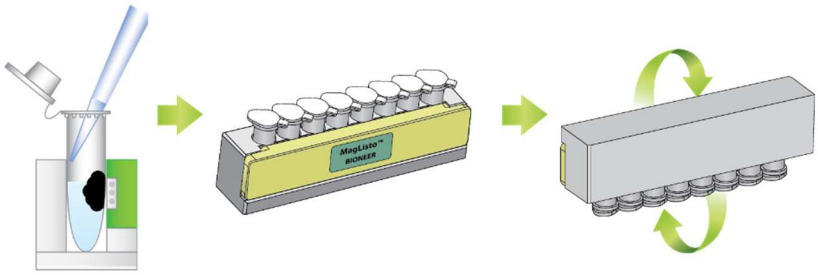


**Figure 5. Washing the beads to remove residual ethanol.** Please refer to the information above.

*** Note:** Direct pipetting of WE Buffer onto the beads, vortexing and/or vigorous shaking of the tubes may release nucleic acid from the beads, which may result in lower RNA yield.

14-B. Drying beads:

Add 700 µl of 80% ethanol, mix thoroughly by vortexing, and repeat the steps 10-11. Completely dry the beads with the tube open at 60°C for at least 5 minutes. Remove the remaining supernatant with a pipette.

15. **(Elution)** Detach the magnet plate from *MagListo*™-2 Magnetic Separation Rack. Add 50-100 µl of ER Buffer to each tube and resuspend nucleic acid by vortexing or pipetting.

16. Incubate at 56-60°C for 1 minute.

17. Attach the magnet plate to *MagListo*™-2 Magnetic Separation Rack and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

18. Without removing the tube from *MagListo*™-2 Magnetic Separation Rack, transfer supernatant containing nucleic acid carefully to a new tube.

19. Discard the tubes with the remaining beads.

*** Note:** Do not reuse the beads.

## Troubleshooting

| Problem | Comments |
|---|---|
| **Low RNA yield or purity** | • **Buffers or other reagents may have been exposed to conditions that reduce their effectiveness.** Store the reagents at room temperature (15-25°C) at all times upon arrival and keep all reagent bottles tightly closed after use to preserve pH and stability and avoid contamination. |
| | • **Poly(A) may not have been completely mixed with VB Buffer.** When adding all dissolved Poly(A) to the VB Buffer, mix completely by vortexing. |
| | • **Ethanol may not have been added to VWM1 Buffer.** Add absolute ethanol (not provided) to the VWM1 Buffer (see “Before You Begin” on page 10) and mix well. Mark VWM1 Buffer bottle label to indicate whether ethanol has been added or not. |
| | • **Reagents and samples may not have been completely mixed.** Always mix the sample tube well after adding each reagent. |
| **High absorbance at 260 nm** | • VB Buffer in the kit needs to be added Poly(A) as RNA carrier for enhancing efficiency of RNA extraction. Because Poly(A) has UV absorbance and it is more abundant than viral RNA, extracted RNA can show high absorbance at 260 nm. For quantification of extracted viral RNA, we recommend amplification. |

## References

Bonham, M. J., & Danielpour, D. (1996). Improved purification and yields of RNA by RNeasy®. *Biotechniques*, *21*(1), 57-60.

Coombs, N.J., Gough, A.C., and Primrose J.N. (1999) Optimisation of DNA and RNA extraction from archival formalin-fixed tissue. *Nucleic Acids Research*, *27*(16), e12.

Reno, C., Marchuk, L., Sciore, P., Frank, C.B., and Hart, D.A. (1997) Rapid isolation of total RNA from small samples of hypocellular, dense connective tissues. *Biotechniques*, *22*(6), 1082-1086.

## Ordering Information

| Description | | Cat. No |
|---|---|---|
| *MagListo*™ 5M Viral DNA/RNA Extraction Kit | 100 reactions | K-3624 |
| | 500 reactions | K-3623 |

## Related Products

| Description | Cat. No |
|---|---|
| Proteinase K Powder | KB-0111 |
| RNase A Powder | KB-0101 |
| *MagListo*™-8Ch Magnetic Separation Rack | TM-1000 |
| *MagListo*™-2 Magnetic Separation Rack | TM-1010 |
| *MagListo*™-2-12h Magnetic Separation Rack | TM-1011 |
| *MagListo*™-15 Magnetic Separation Rack | TM-1020 |
| *MagListo*™-50 Magnetic Separation Rack | TM-1030 |

## Explanation of Symbols

| | | | |
|---|---|---|---|
| LOT Batch Code | Consult Instructions For Use | RUO Research Use Only | Caution |
| Biological Risks | Contains Sufficient for <n> tests | Temperature Limitation | Manufacturer |
| REF Catalog Number | Do not Re-use | Use-by Date | |

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