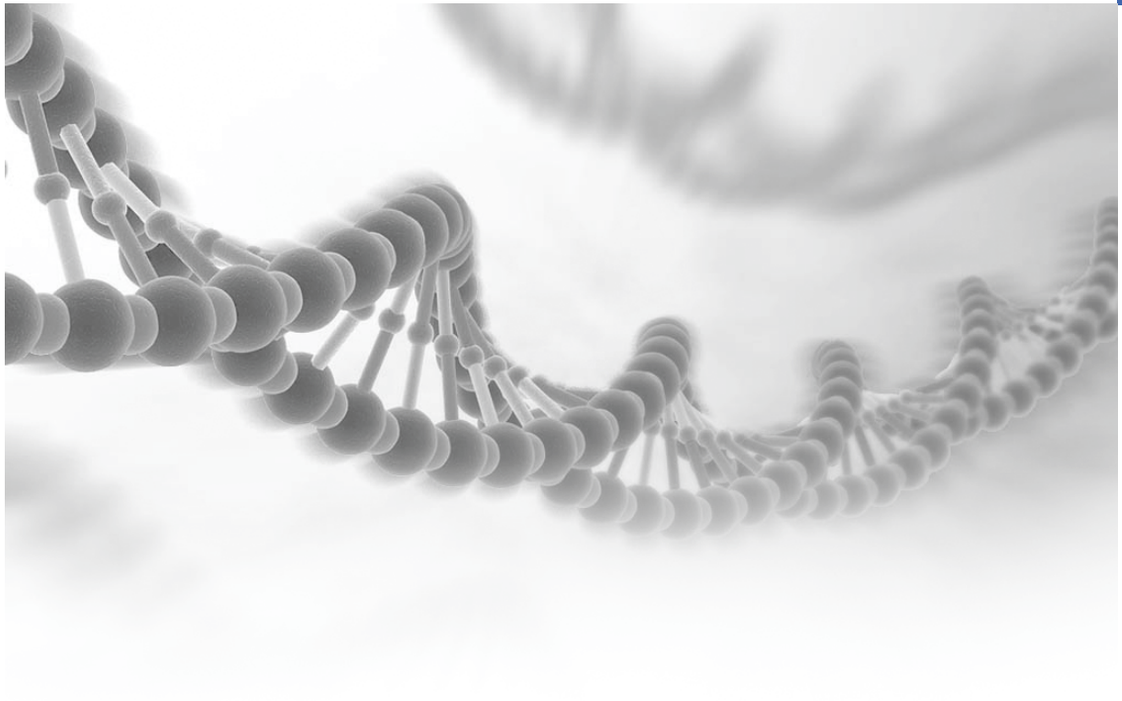
GENETIX BRAND

HANDBOOK

Nucleo-pore®



Viral Nucleic Acid Isolation

☐	**RNASure® Virus Kit**	NP-67705	50 Preps
☐	**RNASure® Virus Kit**	NP-67707	250 Preps

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Nucleo-pore®

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COMPONENTS

Kit Contents

RNASure® Virus Kit

Catalog No.	NP-67705	NP-67707
No of preps	50	250
Lysis Buffer VLB1	35ml	5 x 35ml
Wash Buffer VWB	30ml	2 x 75ml
Wash Buffer VWB3 (Conc)*	12.5ml	3 x 25ml
RNase Free water	5ml	25ml
Elution Buffer VEB	5ml	25ml
Carrier RNA (lyophilised)*	1mg	5 x 1mg
RNASure® Virus Column	50	250
Collection Tube 2ml	150	750
Handbook	1	1

* Please see "Preparation of Reagents"

Reagents, consumables and equipments not provided with the kit

- 95 - 100 % ethanol
- 1.5 ml microcentrifuge tubes
- Proteinase K (for isolation of viral DNA and RNA, see supplementary protocol
- Sterile RNase-free pipette tips
- Manual pipette
- Centrifuge for 1.5ml microcentrifuge tubes
- Equipment for sample disruption and homogenization
- Personal protection equipment (lab coat, gloves, goggles)

SAFETY INSTRUCTIONS

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).

Buffers VLB1and VWB contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.
The following risk and safety phrases apply to components of the RNASure® Virus Kit

Buffer VLB1
Contain Guanidine thiocyanate: R&S Phrases: R20/21/22, S13

Buffer VWB
Contain Guanidine hydrochloride: R&S Phrases: R10-22-36/38, S7-16

R10: Flammable, R20/21/22: Harmful by inhalation, in contact with skin and if swallowed, R36/38: Irritating to eyes and skin, S7: Keep container tightly closed , S13: Keep away from food, drink and animal foodstuffs, S16: Keep away from sources of ignition - No smoking.

INTRODUCTION

Principle and Procedure

RNA viruses are lysed by Lysis Buffer VLB1 whereas, DNA viruses which are relatively more difficult to lyse require Proteinase K digestion. Addition of Carrier RNA improves binding and recovery of low-concentrated viral RNA. The RNA binds to the membrane and contaminants (potential PCR inhibitors) like salts, metabolites and soluble macromolecular cellular components are removed efficiently in two wash steps using two different buffers VWB and VWB3. The nucleic acids can be eluted in low salt buffer or water (RNase free) and are ready-for-use in subsequent downstream applications like PCR, real time PCR etc. High-quality RNA is eluted in a special RNase-free buffer, ready for direct use or safe storage. The purified RNA is free of any contaminants and inhibitors.

Specifications of RNASure® Virus Kits:

RNASure® Virus kits are designed for the rapid preparation of highly pure viral nucleic acids (e.g. HCV, HIV, CMV) from fluid biological samples e.g. plasma, serum, urine, but not blood. Kit provides a fast and efficient way for reliable and high quality of viral nucleic acid (e.g. HCV, HIV, CMV) from diverse range of biological samples e.g. Plasma, Serum, Urine; but this kit cannot be used with blood. RNASure® Virus kit is intended for general laboratory use. RNASure® Virus kit is suitable for 150µL of sample as starting material. Yield & quality of the prepared nucleic acid is suitable for further application like DNA Sequencing, RT-PCR, RNA dot blots, cDNA transcription, Taqman® analysis and array technologies.

The detection limit of certain viruses depends upon the individual procedures. We recommend using internal (low copy) standards as well as positive and negative control to monitor the purification, amplification and detection processes.The procedure is designed to avoid Sample to Sample cross contaminations and allow safe handling of infectious samples.

Starting Material and Typical Yields

Upto 40 µg of RNA can be extracted from upto 150 µl Serum, Plasma, Cell-free biological fluid in 50 µl elution volume using RNASure® Virus Kit

Handling Samples

Viral nucleic acid can be purified from various sources e.g., serum, plasma and urine. Samples may be fresh or frozen, frozen samples should not be thawed more than once. Incubation with buffer VLB1 can be prolonged to dissolve and digest residual cell structures, precipitates and virus particle. However, RNA is sensitive to autolysis and prolonged incubation may decrease the yields. To prepare viral or genomic DNA for forensic or diagnostic application purposes also see our DNASure® Blood/ Tissue kit series which contain different additional protocols for different starting materials.

Preparation and Storage of Reagents

Precaution:

Please wear gloves & safety goggles. All buffers & kit components stored at room temperature (18-25°C) are stable for at least 12 months.

Carrier RNA

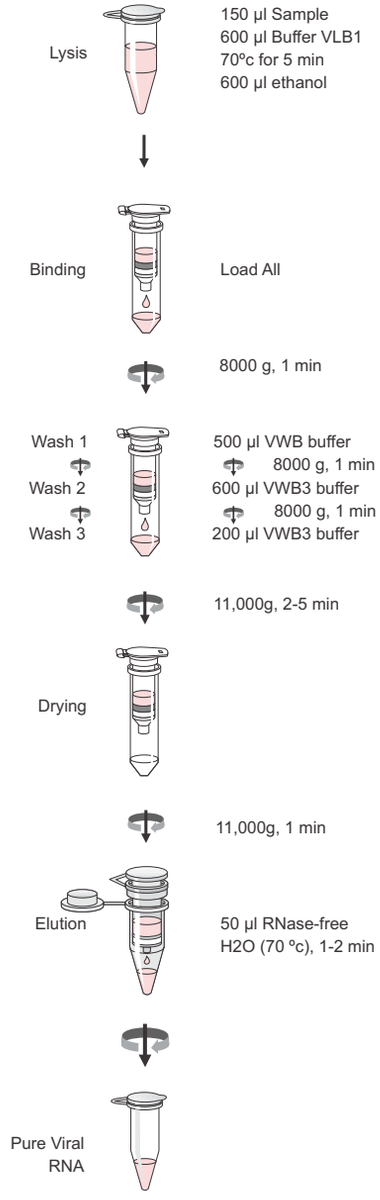
Add 1ml of lysis buffer VLB1 to the complete content of the carrier RNA tube dissolve & transfer it back to the VLB1 bottle. Lysis buffer VLB1 including carrier RNA can be stored at room temperature (18°C-25°C) for 1-2 weeks. Aliquot and store at -20°C for longer periods. Storage at 4°C may cause salt precipitation, preheat at 40°-60°C for 5 minutes to re-dissolve salts.

Note: Do not warm Buffer VLB1 *containing Carrier RNA more than* 4 times.

Buffer VWB3

Reconstitute Buffer VWB3 concentrate by adding, 50ml (Kit NP-67705) and 100ml to each bottle (Kit NP-67707) ethanol (96-100%). Mark the bottle as "Ethanol Added". Buffer VWB3 can be stored at room temperature (18-25°C) for at least one year.

Viral RNA Purification



Protocol for Isolation of viral RNA from cell-free biological fluids using RNASure® Virus kit

Things to do before starting:

- Check if Wash Buffer VWB3 was prepared as per instructions.
- Preheat an aliquot of Elution Buffer VEB / RNase-free H_2O to 70 °C.

Procedure

1. **Take 150µl of sample fluid add 600µl of buffer VLB1 containing Carrier RNA. Mix thoroughly and vortex it. Incubate the mixture at 70°C for 5 min. If the solution is still turbid centrifuge it for 1 min at 11,000 x g** (This will pellet particles and will help preventing clogging of RNASure® Virus column).Take off the supernatant and proceed to Step 2.

2. **Add 600µl of ethanol (96-100%) and mix thoroughly by pulse vortexing (10-15 s).**

3. **Place the RNASure® Virus mini column in collection tubes (2ml) and load 700µl of lysate. Centrifuge it for 1 min at 8,000 x g. Take a fresh collection tubes (2 ml). Load the residual lysis solution onto the column and centrifuge at 8,000 x g for 1min.** Discard the flow-through and put the column into a fresh collection tube (2ml).

4. **Add 500µl of buffer VWB to the RNASure® Virus column and centrifuge for 1 min at 8000 x g, discard the flowthrough.** This step removes contaminant PCR inhibitors.

5. **Add 600µl of buffer VWB3 to the RNASure® Virus column and Centrifuge for 1 min at 8,000 x g.** Discard the flowthrough along with the collection tube. Transfer the column into a fresh collection tube.

6. **Add 200µl of buffer VWB3 & centrifuge for 2-5 min at 11,000 x g this will remove wash buffer VWB3 completely.**

7. **Place RNASure® Virus column into a new sterile 1.5ml microcentrifuge tube (not provided with kit) add 50µlof RNase free H_2O (preheated to 70°C) and incubate for 1-2 min. Centrifuge it at 11,000 x g for 1 min.**
   Note: Nucleic acids are eluted with RNase-free H_2O (pH about 7-8) or slightly alkaline Buffer VEB (5 mM Tris-HCl, pH 8.5). To increase yield second elution step may be performed.

Supplementary Protocol for Isolation of viral RNA and DNA from cell-free biological fluids using RNASure® Virus kit.

Things to do Before starting:

- Check if Wash Buffer VWB3 and Carrier RNA were prepared according to instructions.
- Arrange and reconstitute Proteinase K as per manufacturers protocol.
- Preheat an aliquot of Elution Buffer VEB / RNase-free H_2O to 70 °C.

Procedure

1. **Take 150µl of sample and add 600µl of buffer VLB1 containing carrier RNA, to it add 20µl Proteinase K (20mg/ml stock solution is not provided with the kit). Mix thoroughly using micropipette followed by pulsevortexing for 10-15 seconds. Incubate the mixture at 70°C for 5 min.** Incubation time and temperature are critical for lysis as well as RNA stability If the resulting solution is still turbid, centrifuge the mixture for another 1 min at 11,000 x g to pellet particles, to prevent clogging of the RNASure® Virus Columns.

2. **To the above solution add 600µl of ethanol (96-100%) and mix thoroughly by pulse vortexing (10-15 s).**

3. **Place the RNASure® Virus mini column in collection tubes (2ml) and load 700µl of lysed sample. Centrifuge it for 1 min at 8,000 x g. Load the residual lysis solution onto the column. Centrifuge at 8,000 x g for 1min.** Discard the flow-through and put the column into another new collection tube (2ml). Do not load more than twice.

4. **Add 500µl of buffer VWB to the RNASure® Virus column and centrifuge for 1 min at 8000 x g, discard the flow through.** This step removes contaminant PCR inhibitors.

5. **Add 600µl of buffer VWB3 to the RNASure® Virus column and centrifuge for 1 min at 8,000 x g.** Discard the flow through along with the collection tube. Transfer the column into a fresh collection tube.

6. **Add 200µl of buffer VWB3 & centrifuge for 2-5 min at 11,000 x g this will remove ethanolic buffer VWB3 completely.**

7. **Place RNASure® Virus column into a new sterile 1.5ml microcentrifuge tube (not provided with kit) add 50µlof RNase free H_2O (preheated to 70°C) and incubate for 1-2 min. Centrifuge it at 11,000 x g for 1 min, collect the eluate.**

TROUBLESHOOTING GUIDE

General Problem

Possible cause

- Clogged Membrane

Suggestion(s)

- After plasma lysis centrifuge it to Pellet debris before the addition of ethanol and subsequent loading into the corresponding RNASure® Virus Mini Column.

Problem with Detection

Possible cause

- Ethanol Carryover

Suggestion(s)

- Increase centrifugation time to remove Buffer VWB3 completely.

Possible cause

- Reduced Sensitivity in downstream application

Suggestion(s)

- Change the volume of eluate that is added in PCR/ RT-PCR reaction as incubation time & temperature are critical in lysis step. Generally for sensitive RNA applications room temperature incubation is okay.
- For parallel isolation of both viral RNA & DNA incubation time (5-15 min) and temperature (RT/56°C/72°C) may be adapted for optimal recovery of both nucleic acid.

Small amount or no viral nucleic acids in the eluate.

Possible Cause

- Problem with Carrier RNA

Suggestion(s)

- Carrier RNA not added
- Check for storage condition from Buffer VLB1 with carrier RNA
- Standardize protocols with and without Proteinase K digestion, incubation may be prolonged to 10 min.

Possible Cause

- Viral RNA/DNA Degraded

Suggestion(s)

- Process fresh samples and if required RNase inhibitors may be added to sample. Follow appropriate storing condition for all buffers, if doubt persist use new set of buffer VLB1 & elution Buffer VEB.

ORDERING INFORMATION

Description	Pack Size	Cat. No.
DNASure® Tissue Mini Kit	50 preps	NP-61305
DNASure® Plant Mini Kit	50 preps	NP-79105
DNASure® Plant Mini Kit	250 preps	NP-79107
DNASure® Plant Midi Kit	20 preps	NP-78153
DNASure® Plant Maxi Kit	10 preps	NP-78164
DNASure® Blood Mini Kit	50 preps	NP-61105
DNASure® Blood Mini Kit	250 preps	NP-61107
DNASure® Blood Midi Kit	20 preps	NP-61184
DNASure® Blood Maxi Kit	10 preps	NP-61193
DNASure® Blood FastPure Kit	50 preps	NP-62205
DNASure® Blood FastPure Kit	250 preps	NP-62207
SureSpin® Plasmid Mini Kit	50 preps	NP-37105
SureSpin® Plasmid Mini Kit	250 preps	NP-37107
SureSpin® Plasmid FastPrep Kit	50 preps	NP-47105
SureSpin® Plasmid FastPrep Kit	250 preps	NP-47107
SureSpin® Buffer Set*	1	37107-BS
SurePrep® Plasmid Mini Kit	20 preps	NP-15123
SurePrep® Plasmid Mini Kit	100 preps	NP-15125
SurePrep® Plasmid Midi Kit	20 preps	NP-15143
SurePrep® Plasmid Midi Kit	100 preps	NP-15145
SurePrep® Plasmid Maxi Kit	10 preps	NP-15161
SurePrep® Plasmid Maxi Kit	25 preps	NP-15162
SurePrep® Plasmid Mega Kit	5 preps	NP-15183
SurePrep® Plasmid Giga Kit	5 preps	NP-15191

***SureSpin® Buffer Set**

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

****SureSpin® Buffer Set**

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

Product Warranty

RNASure® Virus Kit components are intended for research purposes only. They are suitable for *in vitro* uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the RNASure® Virus Kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

In accordance with Genetix ISO-certified Quality Management System, each lot of RNASure® Virus Kit is tested against predetermined specifications to ensure consistent product quality.

In no event shall Genetix be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Genetix products to perform in accordance with the stated specifications.

Product claims are subject to change. Therefore please contact our Technical Support Department for updated information on Genetix products.

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