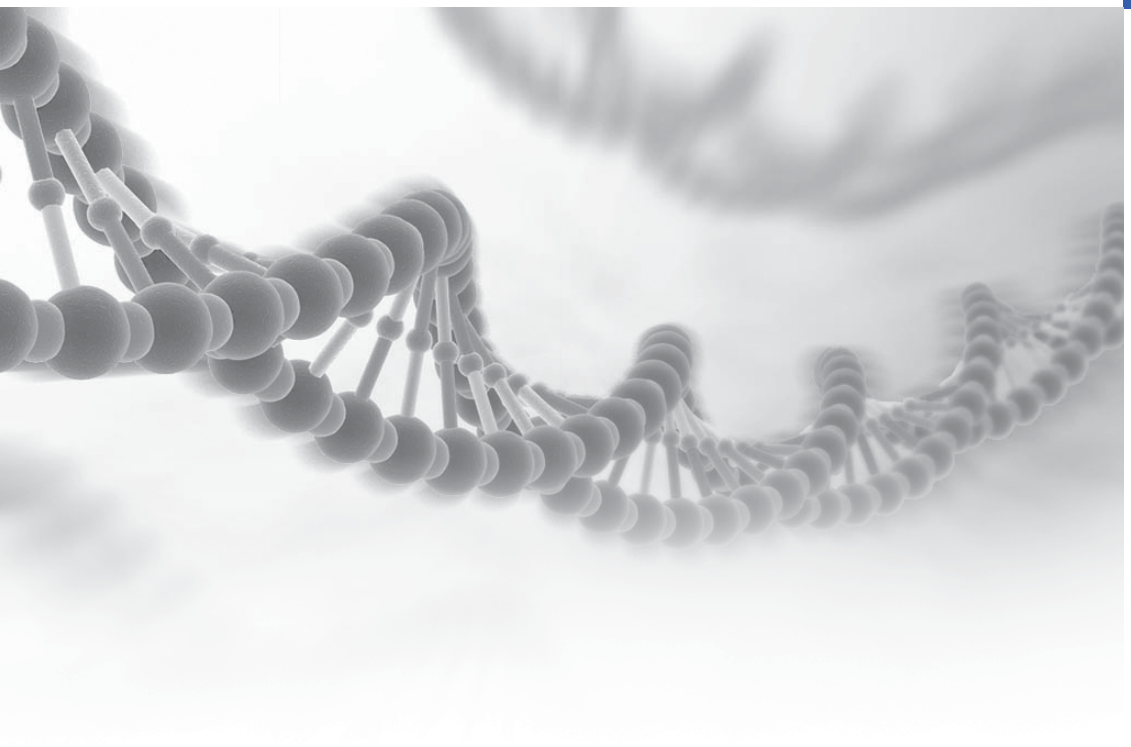
GENETIX BRAND

HANDBOOK

Nucleo-pore®



Nucleopore® Viral RNA Extraction Kit

☐	**Nucleopore® Viral RNA Extraction Kit**	NP - 051223	50 Preps

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Nucleo-pore®

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COMPONENTS

Kit contents

Cat. No.	NP-0151223	
Components	**Quantity**	**Storage**
Buffer NVL	16 ml	Room temperature (15~25°C)
Buffer RB1	22 ml	
Buffer RBW	30 ml	
Buffer RNW	30 ml	
Nuclease-free water	15 ml	
Carrier RNA*	370 ug	
Column micro S with collection tube	50	
1.5 ml microcentrifuge tube	50	

* Reconstitute 70μg of carrier RNA by adding 370μl of Nuclease free water. Also please read "Important Notes" on page 4

Nucleopore® Viral RNA Extraction Kit

Type	Spin
Maximum volume of starting samples	100 ul / prep
Preparation time	~ 15 minutes
Maximum loading volume	750 ul
Minimum elution volume	20 ul

Quality Control

All components in Nucleopore® Viral RNA Extraction kit are manufactured in strictly clean condition, and its degree of cleanness is monitored periodically.
For consistency of product, the quality certification process is carried out from lot to lot thoroughly and only the qualified is approved to be delivered.

Storage condition

All components of Nucleopore® Viral RNA Extraction kit should be stored at room temperature (15~25°C). After reconstitution of carrier RNA with nuclease-free water, it should be stored in aliquots at -20°C for conservation of activity or immediately used for experiments.
Under cool ambient condition, a precipitate can be formed in buffer NVL. In such a case, heat the bottle above 37°C to dissolve completely. Nucleopore® Viral RNA Extraction kit is guaranteed until the expiration date printed on the product label.

Precautions

Buffer NVL, RB1, and RBW contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions

Preventing RNase contamination

Rnase can be introduced accidentally into a RNA preparation. Wear disposable gloves always, because skin often contains bacteria that can be a source of RNase. Use sterile, disposable plasticwares and automatic pipettes reserved for RNA work to prevent cross-contamination with RNase on shared equipment.

Product Description

Nucleopore® Viral RNA Extraction kit provides a convenient method for isolation of RNA and DNA from cell-free fluid, cell-cultrue supernatant, plasma, serum, swab, urine, and virus-infected samples.
Nucleopore® Viral RNA Extraction kit procedures employed the glassfiber membrane technology for the fastest and the most convenient of high purity RNA and DNA isolation, instead of conventional alcohol precipitation or phenol/chloroform extraction.
Nucleopore® Viral RNA Extraction kit buffer system provides the effective binding condition of RNA and DNA to glassfiber membrane and the impurities on the membrane are washed away by two different wash buffers. At last, pure RNA and DNA are eluted by nuclease-free water. Whole procedure takes only 15 minutes and the purified nucleic acid is suitable for PCR, RT-PCR, or any downstream application without further manipulation.
Nucleopore® Viral RNA Extraction kit procedure should be performed at room temperature. The purified nucleic acid should be treated with care because RNA is very sensitive to contaminants, such as RNases, often found on general labware and dust. To ensure RNA-stability, it is recommended to store at 4°C for immediate analysis or to freeze at -70°C for long-term storage.

Important Notes

Before Experiment
Starting material, such as plasma or serum, should be stored at -70°C in aliquots for long term storage. Repeated freezing and thawing of frozen plasma or serum leads to protein precipitation, causing reduced viral titers and subsequently decreased yields of the isolated viral nucleic acid. Besides, protein precipitant will cause clogging of spin column.
Nucleopore® Viral RNA Extraction kit is designed to extract total nucleic acids from samples including virus and host cell. The use of cell-free body fluids is recommended for isolation of viral nucleic acid, and the extraction efficiency can vary depending on the type of virus and sample media.
Provided carrier RNA can help to improve the binding of viral nucleic acids to the spin column especially in the case of very few target nucleic acids in the samples, and protect target nucleic acids from the chance of degradation due to residual RNase activity.

Carrier RNA
This kit is provided with carrier RNA, which can be added at lysis step if required. Carrier RNA enhances binding of nucleic acid to the spin column membrane, especially if there are very few target molecules in the sample.
For purification of nucleic acid from very small amounts of sample, we recommend adding carrier RNA at lysis step. To obtain a solution of 1 ug/ul, add 370 ul of nuclease-free water to the tube containing 370 ug lyophilized carrier RNA. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store at -20°C . Do not freeze-thaw the aliquots of carrier RNA more than 3 times. For one preparation, 7ul of dissolved carrier RNA is required.

Protocol for Nucleopore® Viral RNA Extraction kit

1. **Add 300 ul of buffer NVL and 7 ul of carrier RNA into a tube.**
2. **Transfer upto 100 ul of serum sample into the tube.**
   If the sample volume is less than 100 ul, adjust the volume to 100 ul with PBS.
   In case of large sample volume, increase the amount of buffer NVL and carrier RNA proportionally.
3. **Mix thoroughly by vortexing for 10 seconds.**
   For proper lysis, the complete mix of sample and buffer NVL is essential.
4. **Incubate the mixture for 10 minutes at room temperature.**
5. **Add 350 ul of buffer RB1 to the mixture and mix thoroughly by vortexing for 10 seconds.**
   The volume of buffer RB1 can be adjusted in proportion to the volume of lysate.
   Do not centrifuge at this step. Nucleic acids can be precipitated through centrifugation.
6. **Transfer upto 750 ul of the mixture to a spin column (Column type micro S, white).**
7. **Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.**
   Discard the pass-through and reinsert the spin column back into the same tube.
   If the sample volume exceeds 750 ul, repeat step 6 ~ 7 with the remainder of the sample.
8. **Add 500 ul of buffer RBW to the spin column.**
9. **Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature**
   Discard the pass-through and reinsert the spin column back into the same tube.
10. **Add 500 ul of buffer RNW to the spin column.**
11. **Centrifuge at ≥ 10,000 x g for 30 seconds at room temperature.**
    Discard the pass-through and reinsert the spin column back into the same tube.
12. **Centrifuge at full speed for an additional 1 minute at room temperature to remove residual wash buffer.**
    Transfer the spin column to a new 1.5 ml microcentrifuge tube (provided).
    Residual ethanol may interfere with downstream reactions.
    Care must be taken at this step for eliminating the carryover of buffer RNW.
13. **Add 25 ~ 50 ul of nuclease-free water to the center of the membrane in the spin column.**
    Let it stand for 1 minute.
14. **Centrifuge at ≥ 10,000 x g for 1 minute at room temperature.**
    Purified nucleic acids can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.

TROUBLESHOOTING GUIDE

Low yield

Possible cause
- Poor quality of starting material

Suggestion
- Repeated freezing and thawing should be avoided.

Possible cause
- Low concentration of virus in the sample

Suggestions
- Use more sample. Concentrate the sample volume to 300 ul using a microconcentrator.

Possible cause
- Sample not homogenized completely

Suggestions
- Be sure to incubate for 10 minutes at room temperature after lysis.

For proper lysis, the complete mix of sample and buffer NVL is essential.

Possible cause
- Incorrect elution conditions

Suggestions
- Add nuclease-free water to the center of the spin column membrane and perform incubation for 1 minute before centrifugation.

Possible cause
- Precipitation of buffer NVL

Suggestions
- Storage at low temperature may cause precipitation in buffer NVL. For good result, any precipitate in the buffer should be dissolved completely by incubating the buffer at 37°C(or above) until it disappears.

Possible cause
- Degradation of RNA

Suggestions
- RNase can be introduced during use. Be certain not to introduce any RNases during the procedure or later handling. Keep tubes closed whenever possible during the preparation.

TROUBLESHOOTING GUIDE

Low yield

Possible cause
- Carrier RNA not added

Suggestion
- Add carrier RNA at lysis step. Omission of carrier RNA leads to low purification efficiency.

Possible cause
- Degradation of carrier RNA

Suggestions
- Carrier RNA was not stored at -20°C or afflicted with multiful freeze-thaw cycles. After reconstitution, carrier RNA should be stored in aliquots at -20°C.

Possible cause
- Buffer RBW and RNW used in the wrong order

Suggestions
- Ensure that buffer RBW and RNW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with RNW.

Eluate does not perform well in downstream application

Possible cause
- Residual ethanol remains in eluate

Suggestion
- To remove any residual ethanol included in buffer RNW from spin column membrane, centrifuge again for complete removal of ethanol (step 12).

Possible cause
- Buffer RBW and RNW used in the wrong order

Suggestions
- Ensure that buffer RBW and RNW are used in the correct order in the protocol. If used in the wrong order, perform the last washing step with RNW.

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

ORDERING INFORMATION

Description	Pack Size	Cat. No.
Nucleo-pore® Stool DNA Mini Kit	50	NP-7011D
Nucleo-pore® gRNA Blood Kit	50	NP-0201R
Nucleo-pore® gDNA Urine Kit	20	NP-6030D
Nucleo-pore® Yeast Transformation Kit	120	NP-1002T
Nucleo-pore® DNA Methylation Kit	50	NP-6006D
Nucleo-pore® gDNA Clean-up Kit	200	NP-4304D
Nucleo-pore® Bisulphite DNA Clean-up Kit	50	NP-5205D
Nucleo-pore® gDNA Fungal/Bacterial Mini Kit	50	NP-7006D

Product Warranty

Nucleopore® Viral RNA Extraction 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 Nucleopore® Viral RNA Extraction 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 Nucleopore® Viral RNA Extraction 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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