Ver 1.0

Cat.No. 322-150

# Ribospin™ vRD II

## VIRAL RNA PURIFICATION HANDBOOK

GeneAll

## Customer & Technical Support

Do not hesitate to ask us any question.

We thank you for any comment or advice.

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## Trademarks

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This protocol handbook is included in :

GeneAll® Ribospin™ vRD II (322-150)

Visit www.geneall.com or www.geneall.co.kr for FAQ, QnA and more information.

# INDEX

# KIT CONTENTS

| 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 ea | |
| 1.5 ml microcentrifuge tube | 50 ea | |

* Refer to page 7 for carrier RNA

# Product Specifications

| Ribospin™ vRD II | |
|---|---|
| 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 Ribospin™ vRD II 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 Conditions

All components of GeneAll Ribospin™ vRD II 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. GeneAll Ribospin™ vRD II 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

Ribospin™ vRD II 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.

Ribospin™ vRD II 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.

Ribospin™ vRD II 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.

Ribospin™ vRD II 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.

## 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.

Ribospin™ vRD II 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

# Ribospin™ vRD II

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

| Facts | Possible Causes | Suggestions |
|---|---|---|
| Low yield | Poor quality of starting material | Repeated freezing and thawing should be avoided. |
| | Low concentration of virus in the sample | Use more sample. Concentrate the sample volume to 300 ul using a microconcentrator. |
| | Sample not homogenized completely | Be sure to incubate for 10 minutes at room temperature after lysis.<br>For proper lysis, the complete mix of sample and buffer NVL is essential. |
| | Incorrect elution conditions | Add nuclease-free water to the center of the spin column membrane and perform incubation for 1 minute before centrifugation. |
| | Precipitation of buffer NVL | 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. |
| | Degradation of RNA | 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

| Facts | Possible Causes | Suggestions |
|---|---|---|
| | **Carrier RNA not added** | Add carrier RNA at lysis step. Omission of carrier RNA leads to low purification efficiency. |
| | **Degradation of carrier RNA** | 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. |
| | **Buffer RBW and RNW used in the wrong order** | 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** | **Residual ethanol remains in eluate** | To remove any residual ethanol included in buffer RNW from spin column membrane, centrifuge again for complete removal of ethanol (step 12). |
| | **Buffer RBW and RNW used in the wrong order** | 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

### GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*

| Products | | Size | Cat. No. | Type |
|---|---|---|---|---|
| Plasmid Rapidprep | | 50 | 100-150 | mini / spin |
| | | 200 | 100-102 | |

### GeneAll® *Exprep™* *for preparation of plasmid DNA*

| Products | | Size | Cat. No. | Type |
|---|---|---|---|---|
| Plasmid SV | mini | 50 | 101-150 | spin / vacuum |
| | | 200 | 101-102 | |
| | | 1,000 | 101-111 | |
| | Midi | 26 | 101-226 | spin / vacuum |
| | | 50 | 101-250 | |
| | | 100 | 101-201 | |

### GeneAll® *Exfection™*
*for preparation of highly pure plasmid DNA*

| Products | | Size | Cat. No. | Type |
|---|---|---|---|---|
| Plasmid LE *(Low Endotoxin)* | mini | 50 | 111-150 | spin / vacuum |
| | | 200 | 111-102 | |
| | Midi | 26 | 111-226 | spin / vacuum |
| | | 100 | 111-201 | |
| Plasmid EF *(Endotoxin Free)* | Midi | 20 | 121-220 | spin |
| | | 100 | 121-201 | |

### GeneAll® *Expin™* *for purification of fragment DNA*

| Products | | Size | Cat. No. | Type |
|---|---|---|---|---|
| Gel SV | mini | 50 | 102-150 | spin / vacuum |
| | | 200 | 102-102 | |
| PCR SV | mini | 50 | 103-150 | spin / vacuum |
| | | 200 | 103-102 | |
| CleanUp SV | mini | 50 | 113-150 | spin / vacuum |
| | | 200 | 113-102 | |
| Combo GP | mini | 50 | 112-150 | spin / vacuum |
| | | 200 | 112-102 | |

### GeneAll® *Exgene™* *for isolation of total DNA*

| Products | | Size | Cat. No. | Type |
|---|---|---|---|---|
| Tissue SV | mini | 100 | 104-101 | spin / vacuum |
| | | 250 | 104-152 | |
| | Midi | 26 | 104-226 | spin / vacuum |
| | | 100 | 104-201 | |
| | MAXI | 10 | 104-310 | spin / vacuum |
| | | 26 | 104-326 | |
| Tissue *plus!* SV | mini | 100 | 109-101 | spin / vacuum |
| | | 250 | 109-152 | |
| | Midi | 26 | 109-226 | spin / vacuum |
| | | 100 | 109-201 | |
| | MAXI | 10 | 109-310 | spin / vacuum |
| | | 26 | 109-326 | |

### GeneAll® *Exgene™* *for isolation of total DNA*

| Products | | Size | Cat. No. | Type |
|---|---|---|---|---|
| Blood SV | mini | 100 | 105-101 | spin / vacuum |
| | | 250 | 105-152 | |
| | Midi | 26 | 105-226 | spin / vacuum |
| | | 100 | 105-201 | |
| | MAXI | 10 | 105-310 | spin / vacuum |
| | | 26 | 105-326 | |
| Cell SV | mini | 100 | 106-101 | spin / vacuum |
| | | 250 | 106-152 | |
| | MAXI | 10 | 106-310 | spin / vacuum |
| | | 26 | 106-326 | |
| Clinic SV | mini | 100 | 108-101 | spin / vacuum |
| | | 250 | 108-152 | |
| | Midi | 26 | 108-226 | spin / vacuum |
| | | 100 | 108-201 | |
| | MAXI | 10 | 108-310 | spin / vacuum |
| | | 26 | 108-326 | |
| Genomic DNA micro | | 50 | 118-050 | spin |
| Plant SV | mini | 100 | 117-101 | spin / vacuum |
| | | 250 | 117-152 | |
| | Midi | 26 | 117-226 | spin / vacuum |
| | | 100 | 117-201 | |
| | MAXI | 10 | 117-310 | spin / vacuum |
| | | 26 | 117-326 | |
| Soil DNA mini | mini | 50 | 114-150 | spin |
| GMO SV | mini | 50 | 107-150 | spin / vacuum |
| | | 200 | 107-102 | |

### GeneAll® *GenEx™* *for isolation of total DNA*

| Products | | Size | Cat. No. | Type |
|---|---|---|---|---|
| *GenEx™* Blood | Sx | 100 | 220-101 | solution |
| | | 500 | 220-105 | |
| | Lx | 100 | 220-301 | solution |
| *GenEx™* Cell | Sx | 100 | 221-101 | solution |
| | | 500 | 221-105 | |
| | Lx | 100 | 221-301 | solution |
| *GenEx™* Tissue | Sx | 100 | 222-101 | solution |
| | | 500 | 222-105 | |
| | Lx | 100 | 222-301 | solution |

| Products | Scale | Size | Cat. No. | Type |
|---|---|---|---|---|
| **GeneAll® *GenEx™*** *for isolation of total DNA* | | | | |
| *GenEx™* Plant | Sx | 100 | 227-101 | solution |
| | Mx | 100 | 227-201 | |
| | Lx | 100 | 227-301 | |
| *GenEx™* Plant *plus!* | Sx | 100 | 228-101 | solution |
| | Mx | 50 | 228-250 | |
| | Lx | 20 | 228-320 | |
| **GeneAll® DirEx™ series** *for preperation of PCR-template without extraction* | | | | |
| DirEx™ | | 100 | 250-101 | solution |
| DirEx™ *Fast*-Tissue | | 96 T | 260-011 | solution |
| DirEx™ *Fast*-Cultured cell | | 96 T | 260-021 | solution |
| DirEx™ *Fast*-Whole blood | | 96 T | 260-031 | solution |
| DirEx™ *Fast*-Blood stain | | 96 T | 260-041 | solution |
| DirEx™ *Fast*-Hair | | 96 T | 260-051 | solution |
| DirEx™ *Fast*-Buccal swab | | 96 T | 260-061 | solution |
| DirEx™ *Fast*-Cigarette | | 96 T | 260-071 | solution |
| **GeneAll® RNA series** *for preparation of total RNA* | | | | |
| RiboEx™ | mini | 100 | 301-001 | solution |
| | | 200 | 301-002 | |
| Hybrid-R™ | mini | 100 | 305-101 | spin |
| Hybrid-R™ Blood RNA | mini | 50 | 315-150 | spin |
| Hybrid-R™ miRNA | mini | 50 | 325-150 | spin |
| RiboEx™ LS | mini | 100 | 302-001 | solution |
| | | 200 | 302-002 | |
| Riboclear™ | mini | 50 | 303-150 | spin |
| Riboclear™ *plus!* | mini | 50 | 313-150 | spin |
| Ribospin™ | mini | 50 | 304-150 | spin |
| Ribospin ™ vRD | mini | 50 | 302-150 | spin |
| Ribospin ™ vRD *plus!* | mini | 50 | 312-150 | spin |
| Ribospin ™ Plant | mini | 50 | 307-150 | spin |
| Allspin™ | mini | 50 | 306-150 | spin |

| Products | Scale | Size | Cat. No. | Type |
|---|---|---|---|---|
| **GeneAll® *AmpONE™*** *for PCR amplification* | | | | |
| Taq DNA polymerase | | 250 U | 501-025 | (2.5 U/μℓ) |
| | | 500 U | 501-050 | |
| | | 1,000 U | 501-100 | |
| α-Taq DNA polymerase | | 250 U | 502-025 | (2.5 U/μℓ) |
| | | 500 U | 502-050 | |
| | | 1,000 U | 502-100 | |
| Pfu DNA polymerase | | 250 U | 503-025 | (2.5 U/μℓ) |
| | | 500 U | 503-050 | |
| | | 1,000 U | 503-100 | |
| α-Pfu DNA polymerase | | 250 U | 504-025 | (2.5 U/μℓ) |
| | | 500 U | 504-050 | |
| | | 1,000 U | 504-100 | |
| Hotstart Taq DNA polymerase | | 250 U | 531-025 | (2.5 U/μℓ) |
| | | 500 U | 531-050 | |
| | | 1,000 U | 531-100 | |
| Taq Premix | 96 tubes | 20 μℓ | 521-200 | lyophilized |
| | | 50 μℓ | 521-500 | |
| | | 20 μℓ | 526-200 | solution |
| | | 50 μℓ | 526-500 | |
| α-Taq Premix | 96 tubes | 20 μℓ | 522-200 | lyophilized |
| | | 50 μℓ | 522-500 | |
| | | 20 μℓ | 527-200 | solution |
| | | 50 μℓ | 527-500 | |
| HS-Taq Premix | 96 tubes | 20 μℓ | 525-200 | solution |
| | | 50 μℓ | 525-500 | |
| | | 20 μℓ | 520-200 | lyophilized |
| Taq Premix (w/o dye) | 96 tubes | 20 μℓ | 524-200 | lyophilized |
| dNTPs mix | | 500 μℓ | 509-020 | 2.5 mM each |
| dNTPs set (set of dATP, dCTP, dGTP and dTTP) | | 1 ml x 4 tubes | 509-040 | 100 mM |

* Each dNTPs is available

### GeneAll® *AmpMaster™* *for PCR amplification*

| Products | Scale | Size | Cat. No. | Type |
|---|---|---|---|---|
| Taq Master mix | 0.5 ml x 2 tubes | | 541-010 | solution |
| | 0.5 ml x 10 tubes | | 541-050 | solution |
| $\alpha$-Taq Master mix | 0.5 ml x 2 tubes | | 542-010 | solution |
| | 0.5 ml x 10 tubes | | 542-050 | solution |
| HS-Taq Master mix | 0.5 ml x 2 tubes | | 545-010 | solution |
| | 0.5 ml x 10 tubes | | 545-050 | solution |

### GeneAll® *HyperScript™* *for Reverse Transcription*

| Products | Scale | Size | Cat. No. | Type |
|---|---|---|---|---|
| Reverse Transcriptase | | 10,000 U | 601-100 | (200 U/μℓ) |
| RT Master mix | 0.5 ml × 2 tubes | | 601-710 | solution |
| RT Premix | 96 tubes, | 20 μℓ | 601-602 | solution |
| Onestep RT-PCR Master mix | 0.5 ml × 2 tubes | | 602-110 | solution |
| Onestep RT-PCR Premix | 96 tubes, | 20 μℓ | 602-102 | solution |

## NOTE


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