Real Genomics

Store at room temperature 15°C~25°C

Viral Nucleic Acid Extraction Kit
96-well Viral Nucleic Acid Extraction Kit
Protocol Book

Vacuum System Protocol

■ YVN50 // YVN100

■ YVN96B-2 // YVN96B-4 // YVN96B-10

Ver. 3.0


Real Genomics

Precautions

I) Handling Requirements

- *Do not use a kit after its expiration date has passed.*
- *Some reagents contain the hazardous compounds guanidine thiocyanate or guanidine hydrochloride . Do not let these reagents touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If you spill the reagents, dilute the spill with water before wiping it up.*
- *Do not allow reagents containing guanidine thiocyanate to mix with sodium hypochlorite solution or strong acids. This mixture can produce a highly toxic gas.*

II) Laboratory Procedures

- *Handle all samples and the resulting waste as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator has to optimize pathogen inactivation by the Lysis Buffer or take appropriate measures according to local safety regulations. RBC Bioscience does not warrant that samples treated with Lysis Buffer are completely inactivated and non-infectious. After sample processing is completed, remove and autoclave all disposable plastics, if you worked with potentially infectious sample material.*
- *Do not eat, drink or smoke in the laboratory work area.*
- *Wear protective disposable gloves, laboratory coats and eye protection when handling samples and kit reagents.*
- *Do not use sharp or pointed objects when working with the reagent cartridge, in order to prevent damage of the sealing foil and loss of reagent.*
- *Do not contaminate the reagents with bacteria, virus, or ribonuclease. Use disposable pipettes and RNase-free pipette tips only to remove aliquots from reagent bottles. Use the general precautions described in the literature.*
- *Wash hands thoroughly after handling samples and test reagents.*

III) Waste Handling

- *Discard unused reagents and waste in accordance with country, federal,state and local regulations.*

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Viral Nucleic Acid Extraction Kit

Cat.No. YVN50//YVN100

Store at room temperature 15°C~25°C

Kit Contents

Cat.No. YVN50

50 mini preps / kit

Carrier RNA	1mg*
VB Buffer	30ml
W1 Buffer	25ml
R-Wash Buffer (Concentrated)	25ml**
RNase-free Water	10ml
VB Column Set	50sets

(Provided with 2ml Collection Tubes)

Cat.No. YVN100

100 mini preps / kit

Carrier RNA	2mg*
VB Buffer	60ml
W1 Buffer	50ml
R-Wash Buffer (Concentrated)	25ml**
RNase-free Water	10ml
VB Column Set	100sets

Viruses : Retroviruses, Influenza, Enteroviruses, DNA Viruses etc. (Viral DNA/RNA)
Sample Source: Serum, Plasma, Cell-Culture Supernatants, Cell-Free Body Fluids
Sample size: 200µl
Operation time: 20 mins
Downstream Applications: PCR/ RT-PCR for research and molecular assays

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* Dissolve the Carrier RNA with 1ml of RNase-free water and transfer the solution into VB Buffer prior to initial use. Store the Carrier RNA added VB Buffer at 4°C.

** Add 100ml ethanol (96-100%) to R-Wash Buffer prior to initial use.

Important note on DNA/RNA preference

DNA and RNA will co-purify when utilising this kit therefore it is useful for extraction of both DNA and RNA based viruses. To reduce contaminating DNA during RNA virus extraction it is essential to only use cell-free samples. The additional of Carrier RNA is not necessary for DNA virus samples. The kit is optimised for cell-free samples only. For viral extraction from infectious sample like buccal swabs or other specimens utilize Genomic DNA Extraction Kit (YGB series) or Total RNA Extraction Kit (YRB series).

Description

The Viral Nucleic Acid Extraction Kit is specially designed for purification of viral RNA/DNA from cell-free samples. The method utilises detergents and a chaotropic salt to lyse the virus particles. Nucleic acid in the chaotropic salt solution binds to the glass fiber matrix of the columns(1). Carrier RNA enhances recovery of viral RNA in low-titer samples. Following washing off of contaminants, the purified nucleic acid is eluted with RNase-free water. The entire procedure can be completed in 20 minutes and the purified nucleic acid is ready for RT-PCR and PCR.

Quality Control

The quality of Viral Nucleic Acid Extraction Kit is tested on a lot-to-lot basis. All components are tested separately.

Product intended USe (Research/Clinical Application)

This product is offered as a general purpose item and guaranteed for research only. RBC Bioscience Corporation offers no specific claim to identify any specific organism for clinical use (diagnostic, prognostic, therapeutic or blood banking). It is the user's responsibility to validate for any particular usage. The Viral Nucleic Acid Extraction Kit may be used in clinical diagnostics laboratory systems for molecular assays after the laboratory has validated their complete system as required by CLIA'88 regulations in the U.S. or local equivalents in other countries. All due care and attention should be exercised in handling this product.

Reference

Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Note

* *Use sterile, RNase-free pipet tips and microcentrifuge tube. *Wear a lab coat and disposable gloves to prevent RNase contamination.*

Protocol (Addition of Carrier RNA is optional)

Dissolve the Carrier RNA with 1ml of RNase-free water and transfer the solution into VB Buffer prior to initial use.
Store the Carrier RNA added VB Buffer at 4°C.

Addtional requirements:
95%Ethanol
1.5ml microcentrifuge tube(RNase-free)
PBS (If samplevolume<200µl)

Lysis

1. *Transfer 200 µl sample (serum, plasma, body fluids, and cell culture supernatant) into a microcentrifuge tube (not provided). If sample volume is less then 200µl, adjust sample volume to 200 µl with PBS (notprovided).*
2. *Add 400µlof VBBuffer (Carrier RNA added) to the sample, mix by vortexing.*
3. *Incubate at room temperature for10minutes.*

Binding

4. *Place a VB Column in a 2ml Collection Tube.*
5. *Add 500µl of 95% ethanol to the sample lysate and mix immediately by votexing.*
6. *Apply 600µl of ethanol-added mixture from previous step to the VB Column.*
7. *Centrifuge at 10,000 x g (13,000 rpm) for 1 minute.*
8. *Discard the flow-through and apply any remaining mixture from step 1 to the same VB Column.*
9. *Centrifuge at 10,000 x g (13,000 rpm) for 1 minute.*
10. *Discard the flow-through and return the VB Column back to the Collection Tube.*

Wash

11.Add 400 µl of W1 Buffer into the VB Column.
12.Centrifuge at 10,000 x g (13,000 rpm) for 30second.
13.Discard the flow-through and return the VB Column back to the Collection Tube.
14.Add 600µl of R-Wash Buffer (ethanol added) into the VB Column.
15.Centrifuge at 10,000 x g (13,000 rpm) for 30second.
16.Discard the flow-through and return the VB Column back to the Collection Tube.
17.Centrifuge at 10,000 x g (13,000 rpm) for 3 minutes to dry the column matrix.

Nucleic Acid Eluti

18.Place dried VB Column in a clean microcentrifuge tube (RNase-free, not provided)
19.Apply 50µl of RNase-free Water into the center of the column matrix.
20.Stand for 3 minutes until water is fully absorbed by the matrix.
21.Centrifuge at full speed for 1 minute to elute purified nucleic acid. Eluted nucleic acid is free of inhibitors, nucleases, proteins and other impurities and ready for direct application in PCR and RT-PCR or other molecular assays.

Centrifuge/Vacuum System

96-well Viral Nucleic Acid Extraction Kit

Cat.No. YVN96B-2//YVN96B-4//YVN96B-10

Store at room temperature 15°C~25°C

Kit Contents

Cat.No. YDF96B-2
Validation Kit

Viral Nucleic Acid Binding Plate	2 plates
96-well 2ml Sample Plate	4 plates
96-well PCR Plate	2 plates
Sealing Film	5 sheets
VB Buffer	100ml
W1 Buffer	80ml
R-Wash Buffer	25ml
RNase-Free Water	30ml
Carrier RNA	2mg x 2

Cat.No. YDF96B-4
Validation Kit

Viral Nucleic Acid Binding Plate	4 plates
96-well 2ml Sample Plate	8 plates
96-well PCR Plate	4 plates
Sealing Film	10 sheets
VB Buffer	240ml
W1 Buffer	200ml
R-Wash Buffer	100ml
RNase-Free Water	60ml
Carrier RNA	2mg x 4

Cat.No. YDF96B-10
Validation Kit

Viral Nucleic Acid Binding Plate	10 plates
96-well 2ml Sample Plate	20 plates
96-well PCR Plate	10 plates
Sealing Film	25 sheets
VB Buffer	480ml
W1 Buffer	440ml
R-Wash Buffer	100+50ml
RNase-Free Water	120ml
Carrier RNA	2mg x 8

Viruses : Retroviruses, Influenza, Enteroviruses, DNA Viruses etc. (Viral DNA and Viral RNA)
Sample Source: Serum, Plasma, Cell-Culture Supernatants, Cell-Free Body Fluids
Sample size: 200 µl
Operation time: 30 mins
Downstream Applications: PCR/ RT-PCR for research and molecular assays

* Add 4 times volume of Ethanol (96-100%) to 1 volume of R-Wash Buffer before initial use.
** Dissolve each vial of Carrier RNA with 1ml of RNase-Free Water and transfer the solution into VB Buffer prior to initial use.
Store the Carrier RNA added VB Buffer at 4°C.

Description

RBC Bioscience 96-well Viral Nucleic Acid Extraction Kit is based on a reliable convenient silica format and is compatible with vacuum protocol. It contains all the necessary plates, buffers and accessories for easy use straight from the box. DNA and RNA will co-purify when utilising YVN96B system, it is useful for extraction of both DNA and RNA based viruses. To reduce contaminating DNA during RNA virus extraction, it is essential to only use cell-free samples.

Principle

RBC Bioscience 96-well Viral Nucleic Acid Extraction Kit is specially designed for muti-well purification of viral DNA/RNA from cell-free samples. The method utilises detergents and chaotropic salt to lyse the virus particles. Nucleic acid in the chaotropic salt solution binds to the glass fiber matrix of the plates. Carrier RNA enhances recovery of viral RNA in low-titer samples. Following washing off of contaminants, the purified nucleic acid is eluted with RNase-Free Water. The entire procedure can be completed in 30 minutes and the purified nucleic acid is ready for RT-PCR and PCR or other molecular assays.

Product Intended Use (Research/Clinical Applications)

This product is offered as a general purpose item and guaranteed for research only. RBC Bioscience Corporation offers no specific claim to identify any specific organism for clinical use. It is the user's responsibility to validate for any particular usage. The YVN96B series kit may be used in clinical diagnostics laboratory systems for molecular assays after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S.

Vacuum System

RBC 96 Vacuum Manifold or any standard 96/384 microplate vacuum manifold. The vacuum protocol is ideal for benchtop operation and a lower cost solution than centrifuging. However, due to di erences in vacuum pressure between various manifold, vacuum systems and samples, it may be neccessary to optimise this step. RBC recommend a vacuum pressure starting from 10 inches Hg up to maximum 20 inches Hg to ensure all solution passes through each well. In practice all the wells should pass through solution within 3-5 minutes. The yields will show little variation even if some wells pass the solution through at varying speeds (some wells may process very rapidly). Maintain vacuum until the solution fully passes through each well.

Vacuum Protocol

Dissolve each vial of Carrier RNA with 1ml of RNase-Free Water and transfer the solution into VB Buffer prior to initial use. Store the Carrier RNA added VB Buffer at 4℃.
Add 4 times volume of Ethanol (95-100%) to 1 volume of R-Wash Buffer before initial use.

Additional requirements:
95% Ethanol
PBS (If sample volume < 200 µl)

Lysis

1. *Transfer 200 µl sample (serum, plasma, body fluids and cell culture supernatant) into each well of the 96-well 2 ml Sample Plate. If sample volume is less than 200 µl, adjust sample volume to 200 µl with PBS).*
2. *Add 400 µl of VB Buffer (Carrier RNA added).*
3. *Incubate at room temperature for 10 minutes.*
4. *Add 500 µl of 95% ethanol to the sample lysate.*
5. *Mix tenderly by votexing.*

Binding

6. *Place a Viral Nucleic Acid Binding Plate on top of the vacuum manifold. Place a waste tray or the provided 2ml Sample Plate underneath to collect waste.*
7. *Transfer the mixture into each well of the Viral Nucleic Acid Binding Plate.(max 300µl).*
8. *Set vacuum manifold to 10-20 inches Hg for 3-5 minutes until wells have emptied. Refer to vacuum notes above for detailed instructions.*

Wash

9. Add 300 µl of W1 Buffer to each well of the Viral Nucleic Acid Binding Plate.
10. Set vacuum to 10-20 inches Hg for 3-5 minutes until wells have emptied.
11. Add 300 µl of R-Wash Buffer (ethanol added) to each well of the Viral Nucleic Acid Binding Plate to wash again.
12. Set vacuum to 10-20 inches Hg for 3-5 minutes until wells have emptied.
13. repeat Step11-12 again.
14. Set vacuum for another 5 minutes to remove residual ethanol.

Nucleic Acid Elution

15. Place the Viral Nucleic Acid Binding Plate on top of the vacuum manifold. Place the provided 96-well PCR Plate on top of the 2ml Sample Plate as a support plate, then place both inside the vacuum manifold. The Viral Nucleic Acid Binding plate will elute directly into the 96-well PCR Plate.
16. Add 50 µl of RNase-Free Water to the center of each well of the Viral Nucleic Acid Binding Plate.
17. Allow to stand for 3 minutes until the RNase-Free Water has been absorbed by the matrix.
18. Set vacuum to 10-20 inches Hg for 1-2 minutes until wells have fully emptied and all elute is in the 96-well PCR Plate.
19. Seal with Sealing Film or parafilm and store at 4℃ or -20℃.
20. Eluted nucleic acid is free of inhibitors, nucleases, proteins and other impurities and ready for direct
21. application in PCR and RT-PCR or other molecular assays.


Sample Process
Bind
Wash –> Elute
Seal and Store

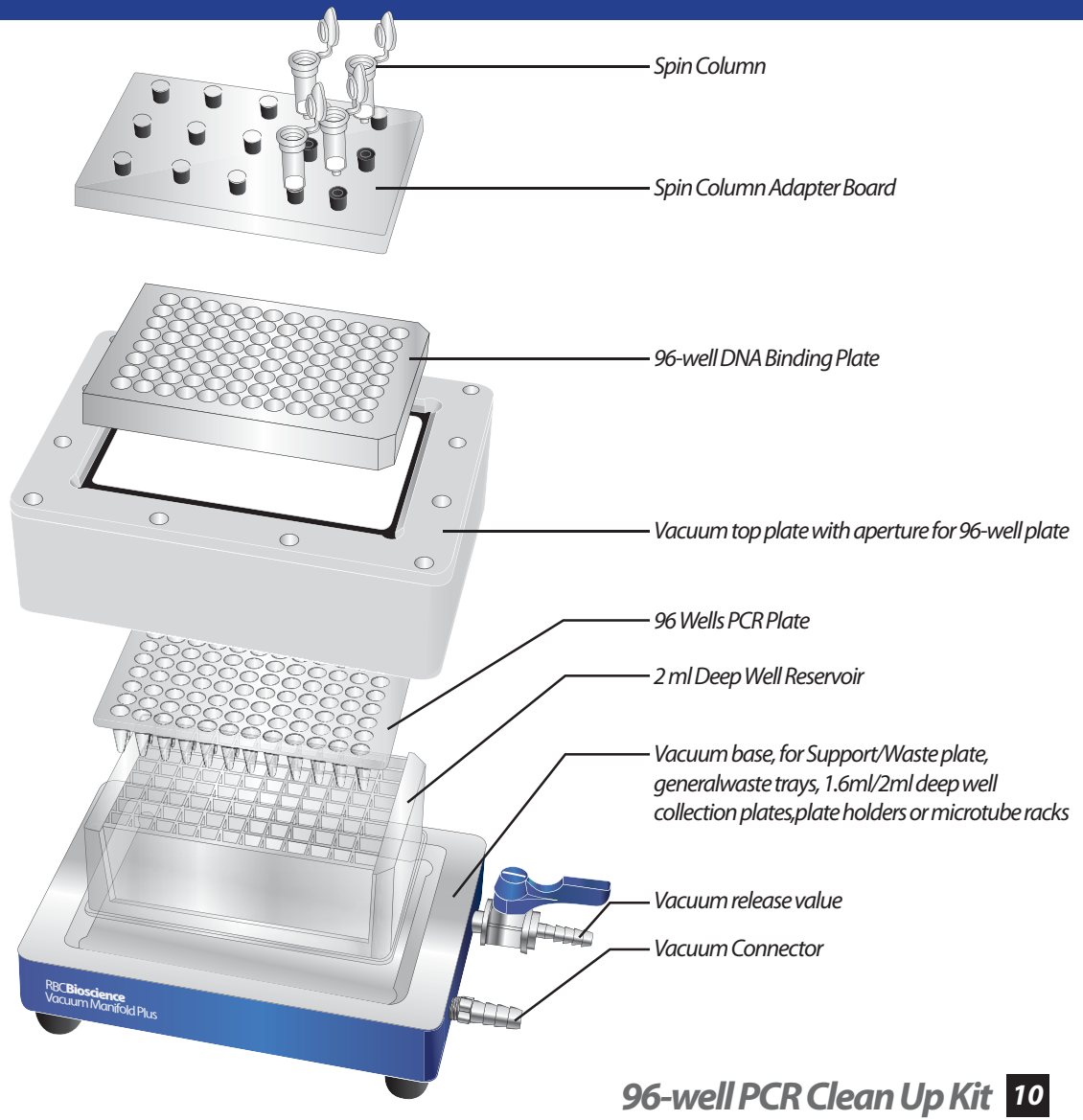
Multi Well Vacuum Manifold Plus
Cat.No. YVM96P

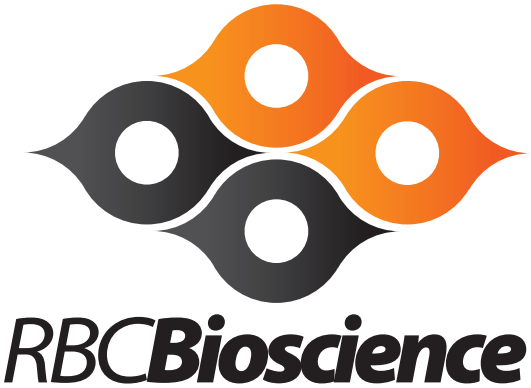
Multi Well Vacuum Manifold Plus

The multi well plate vacuum manifold is an aluminum manifold that has been designed and optimized for the vacuum

filtration of the 96-well filter plates.

* *Comes complete with the necessary O-ring and gasket. The control block includes the vacuum pressure gauge, vacuum metering valve, vacuum release valve, and the 1/4 inch hose barb for vacuum line attachment.*
* *Included with the vacuum manifold unit is a spacer block designed to accommodate standard 350µl receiver plates. The spacer block has been optimized to reduce the space between the receiver plate and the filter plate during vacuum filtration.*

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