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PHASiFY™ VIRAL

RNA Extraction Kit

for VTM Samples (incl. PBS and Saline)
100 reactions

Σ 100

USER MANUAL

IVD

REF 1220200

PHASE Scientific Int'l Ltd.

www.phasescientific.com

PHASESCIENTIFIC

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INTENDED USE

The PHASIFY™ VIRAL RNA Extraction Kit uses the PHASIFY™ proprietary liquid extraction technology to isolate and purify viral RNA from biological specimens collected and stored in Viral Transport Medium (VTM), which includes Phosphate-buffered Saline (PBS) and saline.

The product is intended to be used by professional users, such as clinical or laboratory personnel trained in molecular biological techniques.

The RNA sample extracted using PHASIFY™ VIRAL RNA Extraction Kit is intended for use with downstream *in vitro* diagnostic applications, such as amplification or other enzymatic reactions.

SUMMARY AND EXPLANATION

The PHASIFY™ VIRAL RNA Extraction Kit purifies viral RNA for use in downstream analysis such as RT-qPCR assay or other nucleic acid assay. Viral RNA can be purified from nasal, nasopharyngeal or oropharyngeal swab specimens in VTM, PBS, and saline.

PRINCIPLES OF THE PROCEDURE

The PHASIFY™ technology is a ground-breaking nucleic acid purification technique based on a proprietary liquid extraction mechanism. The PHASIFY™ VIRAL RNA Extraction Kit is uniquely designed to purify RNA from viruses collected and stored in Viral Transport Medium (VTM), which includes Phosphate-buffered Saline (PBS) and saline.

The sample is lysed in highly denaturing conditions to inactivate RNases and isolate RNA. Then the RNA is extracted and purified in a few simple steps.

The novel method significantly improved sample quality by enabling high sample input (up to 600 µL) and flexible resuspension volume (as low as 10 µL) to maximize final sample concentration. High-quality RNA can be resuspended in any DNase / RNase-free buffer of the customers' choice to best fit their downstream analysis protocols. The extracted RNA is ready for direct use or storage.

All buffers and reagents are RNase-free.

Sample Volumes

The PHASIFY™ VIRAL RNA extraction procedures are optimized for 600 µL sample input volume. Samples with smaller volume should be adjusted to 600 µL by adding Solution A before loading.

Lysis

For optimal isolation of viral RNA, a proprietary lysis buffer is designed to lyse the virus and inactivate RNases in the sample simultaneously.

Extract and Concentrate

After RNA is released from the virus, it is concentrated and precipitated into a small pellet through our unique liquid extraction mechanism, which enables high yield of viral RNA.

Removal of Contaminants

While the viral RNA is concentrated into a pellet, most contaminants in the sample are removed with the supernatant. Additional contaminants are removed from the pellet with two alcohol-based wash steps. These steps maximize RNA purity and minimize inhibition of downstream analysis due to contamination.

Resuspension with Buffer

Precipitating RNA into a visible pellet provides the most flexibility regarding the final elution. The pellet can be resuspended in a buffer volume as low as 10 µL to maximize the RNA concentration or in a larger volume for replicate analysis. In addition, the PHASIFY™ VIRAL RNA Extraction Kit allows the customer to use a resuspension buffer that is most appropriate for his/her downstream analysis or storage needs.

Cellular DNA Contamination

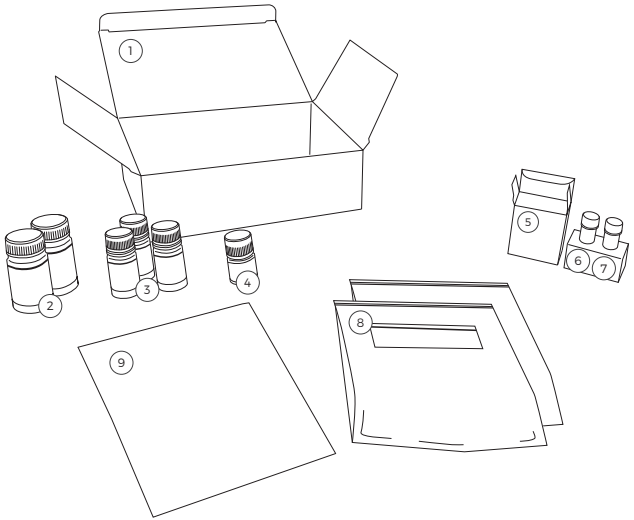
The PHASIFY™ VIRAL RNA Extraction Kit is not designed to separate RNA from DNA, and both may be purified in parallel if present in the sample.

Addition of internal controls

When using the PHASIFY™ VIRAL RNA Extraction Kit protocol in combination with commercially available amplification systems, internal control RNA should be added to the lysis mixture tube before loading VTM.

KIT COMPOSITION (VTM SAMPLE INPUT)

Ref. No. 1220200



PHASIFY™ VIRAL RNA Extraction Kit (100 reactions)

1. RT Box
2. Solution A
3. Solution D1
4. Solution B2
5. COLD Box
6. Powder B1
7. Solution D2
8. Solution C
9. Quick Start Guide

CONTENTS OF KIT PVR.108

Content	Quantity	Storage
RT Box		
Solution A Solution B2 Solution C Solution D1	2 x 24 mL 7.5 mL 100 x 40 µL 3 x 14 mL	15-30˚C
COLD Box		
Powder B1 Solution D2	25 mg 230 µL	4˚C or below

EQUIPMENT & REAGENTS NOT INCLUDED

In addition to the PHASIFY™ VIRAL RNA Extraction Kit, the following items are required:

Equipment & Materials
Empty microcentrifuge / conical tube Microcentrifuge capable of 4,300 x g Vortex-mixer Pipettes (adjustable) Filtered pipette tips (Sterile)
Reagents
40% (v/v) isopropanol (molecular grade) 100% isopropanol (molecular grade) 70% (v/v) ethanol (molecular grade) Resuspension buffer

Note: All reagents and materials should be DNase / RNase-free.

WARNINGS AND PRECAUTIONS

For *In Vitro* Diagnostic Use.

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. When dealing with viral transport media containing live virus, it is important to wear appropriate personal protective equipment (PPE). For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.phasescientific.com, where you can find, view, and print the SDSs for the PHASIFY™ VIRAL RNA Extraction Kit.

If liquid containing the extraction kit buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

RNA is extremely sensitive to RNases and should always be prepared with care. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Change gloves and perform appropriate disinfection procedure after exposure or even suspicious exposure to contamination.

Do not add bleach directly to waste containing Buffer B2, ethanol or isopropanol, as the reaction can result in the release of toxic chemicals, which include chlorine gas or chloroform.

Liquid waste must be considered infectious and should be handled and discarded according to local safety regulations.

The following hazard and precautionary statements apply to components of the PHASIFY™ VIRAL RNA Extraction Kit.

Powder B1

Contains: Proteinase. Danger! Causes mild skin irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. Avoid breathing dust / fume / gas / mist / vapors / spray. Dispose of contents / container to an approved waste disposal plant. If experiencing respiratory symptoms: Call a POISON CENTER or doctor / physician. IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. Wear respiratory protection.

Solution B2

Contains: Denaturant and detergent. Warning, Danger! Harmful if swallowed or if inhaled, causes skin irritation, serious eye irritation and damage. Harmful to aquatic life with long lasting effects. Avoid breathing dust / fume / gas / mist / vapours / spray. Wear eye protection / face protection. Avoid release to the environment. IF SWALLOWED: call a POISON CENTER / doctor if you feel unwell. Rinse mouth. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER / doctor if you feel unwell. IF IN EYES: Rinse cautiously with water for several minutes. If eye irritation persists get medical attention.

Solution D1

Contains: Contains: Iodine ionic compound. Warning! Causes skin and serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes.

REAGENT STORAGE AND HANDLING

The RT Box should be stored at room temperature, between 15-30°C, and away from direct sunlight. The COLD Box which contains Powder B1 and Solution D2 should be stored at 4°C or below.

Solution D1 should be stored protected from light. It can be kept at 15-30°C for 6 months or until the stated expiry date on the package label, but do not use the solution once it turns yellow.

Refer to individual components for stated expiry and storage conditions.

SPECIMEN STORAGE AND HANDLING

Swabbed samples in VTM should be stored at -20°C or below. Samples should be equilibrated to room temperature (15-25°C) before starting the extraction.

IMPORTANT POINTS BEFORE STARTING

Please take a few moments to read this handbook carefully before beginning your extraction.

All steps of the PHASIFY™ VIRAL RNA extraction protocol should be performed smoothly and continuously at room temperature. The PHASIFY™ VIRAL RNA extraction procedure is not designed to separate RNA from DNA.

PREPARATION

Things to do before starting

1. **Solution B1:**
   Add 875 µL DNase / RNase-free water into one vial of Powder B1 (25 mg) and mix well. Store at 4˚C.
2. **Master Mix B:**
   Combine the reagents on the Master Mix B Formulation table found on page 5 of this user manual.
3. **Master Mix D:**
   Combine the reagents on the Master Mix D Formulation table found on page 6 of this user manual.

   Note: The Master Mix Solutions should be made right before extraction and should not be stored for later use.

Master Mix B:

Combine the following reagents for the number of samples required in a microcentrifuge tube or conical tube. To minimize bubble formation, mix the components well by inverting the tube, do not vortex. Prepare fresh on the same day as the extraction and refer to the table below for the amount of components needed per experiment (table values include 10% greater volume than that required for the total number of extractions). For any specific number of reactions not listed in the table, calculate volumes using the following equations:

Volume of **Solution B1** to be added (µL) = No. of reactions x **6.6 µL**
Volume of **Solution B2** to be added (µL) = No. of reactions x **71.5 µL**

Master Mix B Formulation (with 10% Extra Volume)

No. of reactions processed per experiment	Solution B1 (µL)	Solution B2 (µL)
1	6.6	71.5
5	33	357.5
10	66	715
15	99	1072.5
20	132	1430
25	165	1787.5
30	198	2145
35	231	2502.5
40	264	2860
45	297	3217.5
50	330	3575
55	363	3932.5
60	396	4290
65	429	4647.5
70	462	5005
75	495	5362.5
80	528	5720
85	561	6077.5
90	594	6435
95	627	6792.5
100	660	7150

NOTE: The Master Mix Solution should be made right before extraction and should not be stored for later use.

Master Mix D:

Combine the following reagents for the number of samples required in a microcentrifuge tube or conical tube. Gently vortex the tube to mix. Prepare fresh on the same day as the extraction and refer to the table below for the amount of components needed per experiment (table values include 10% greater volume than that required for the total number of extractions). For any specific number of reactions not listed in the table, calculate volumes using the following equations:

Volume of **Solution D1** to be added (mL) = No. of reactions x **0.396 mL**
Volume of **Solution D2** to be added (µL) = No. of reactions x **2.2 µL**

Master Mix D Formulation (with 10% Extra Volume)

No. of reactions processed per experiment	Solution D1 (mL)	Solution D2 (µL)
1	0.396	2.2
5	1.98	11
10	3.96	22
15	5.94	33
20	7.92	44
25	9.90	55
30	11.88	66
35	13.86	77
40	15.84	88
45	17.82	99
50	19.80	110
55	21.78	121
60	23.76	132
65	25.74	143
70	27.72	154
75	29.70	165
80	31.68	176
85	33.66	187
90	35.64	198
95	37.62	209
100	39.60	220

NOTE: The Master Mix Solution should be made right before extraction and should not be stored for later use.

PROTOCOL

Note: Unless specified otherwise, perform the experiment at room temperature

1. Add 71 µL **Master Mix B** into a provided **Solution C** tube (briefly centrifuge the Solution C tube before use to collect the droplets on the tube cap). Then add into the tube 140 - 600 µL **VTM sample**. If less than 600 µL of VTM sample is added, top up the sample to 600 µL with **Solution A**. (e.g. if the VTM sample input is 140 µL, add 460 µL Solution A to top up to 600 µL.)
2. Vortex the tube vigorously at max speed for at least 20 sec until the mixture turns turbid, and then briefly centrifuge. Incubate at room temperature for 10 min.
3. Add 362 µL **Master Mix D** and then 850 µL 100% Isopropanol (not provided) to the tube from Step 2.

   ***NOTE**: The liquid level is close to the tube rim. Be careful when closing.*
4. Vortex until homogenous and incubate at room temperature for 5 min.
5. Centrifuge at max speed for 10 min. Max speed should be no lower than 4,300 x g.
6. Discard all supernatant. Add 1 mL 40% Isopropanol (not provided).

   ***NOTE**: Do not break the pellet at the bottom.*
7. Centrifuge at max speed for 2 min. Max speed should be no lower than 4,300 x g.
8. Discard all supernatant. Add 1 mL 70% Ethanol (not provided).

   ***NOTE**: Do not break the pellet at the bottom.*
9. Centrifuge at max speed for 2 min. Max speed should be no lower than 4,300 x g.
10. Discard all supernatant. Dry the pellet at room temperature for at least 10 min until completely dried.

    ***NOTE**: Do not break the pellet at the bottom. The pellet may disappear during the drying period. Marking the pellet prior to the drying period is recommended.*

    IMPORTANT: Underdrying the pellet will inhibit downstream analysis. Make sure there are no visible droplets in the tube and around the pellet.

11. Resuspend the pellet in at least 10 µL of a resuspension buffer (not provided). Add buffer directly to the dry pellet. Pipette-mix up and down 30 times or more and avoid contacting the walls of the tube.

    IMPORTANT: Incomplete resuspension of the pellet may result in lower yield.

    For immediate use: Resuspend in DNase/RNase-free water and keep on ice.

    For long-term storage: Resuspend in common buffer compatible with downstream analysis. Store at -20°C or lower. Extracted RNA is stable up to 1 year at -80°C or below. Avoid multiple freeze-thaw cycles.

QUALITY CONTROL

PHASIFY™ VIRAL RNA Extraction Kit is produced in accordance with PHASE Scientific's Quality Management System. Each lot is tested against predetermined specifications to ensure consistent product quality.

LIMITATIONS

The system performance has been established using VTM for isolation of viral RNA.

It is the user's responsibility to validate system performance for any procedures used in their laboratory, which are not covered by the PHASIFY™ performance studies. To minimize the risk of a negative impact on the diagnostic results, adequate controls for downstream applications should be used.

Any diagnostic results that are generated must be interpreted in conjunction with other clinical or laboratory findings.

TECHNICAL ASSISTANCE

If you have any queries regarding PHASIFY™ VIRAL RNA Extraction Kit, please do not hesitate to contact us by:

Email:	phasify@phasesci.com
Service hotline:	+1(657) 296 6106 (US)
	+(852) 9135 2570 (Hong Kong)

The PHASE Technical Service Team will help to solve your concerns with our best effort.

TROUBLESHOOTING

Observation	Comments and Recommended Actions
My RNA recovery / yield is lower than I expected.	RNA recovery and yield are influenced by numerous factors. Below are potential reasons and recommended actions: • The VTM sample contains low viral load. Increase the amount of VTM sample input up to 600 µL. • There may be insufficient mixing of the solutions at the vortexing steps (Steps 2 and 4). Vortex vigorously and thoroughly until homogenous. • Incorrect preparation of Master Mix B and / or D. Strictly follow the table on page 5 and 6 for the correct amount of each component. • Incomplete drying of pellet at Step 11. Remaining alcohol surrounding the pellet will affect RNA detection. Increase the drying time. • Incomplete resuspension of pellet. Fully resuspend pellet by pipetting up and down at least 30 times. • Immediately store the sample tube on ice after adding resuspension buffer to avoid RNA degradation.
The pellet looks abnormal after Step 9 (e.g. impurities, gel-like pellet).	• Perform extra 70% ethanol washing step by repeating steps 8 and 9 before proceeding to step 10 to remove more impurities. Note: increasing the number of washing steps may lower the RNA recovery.
The pellet was disturbed and broken during attempt to discard the supernatant in Steps 6,8, and 10.	• If all the supernatant cannot be removed due to a loose or broken pellet, centrifuge the sample again at max speed (no lower than 4,300 x g) for 2 min, then attempt to remove all supernatant. Removing all supernatant is especially important at Step 10.
Pellet is difficult to resuspend.	• After adding resuspension buffer, resuspend the pellet by pipetting up and down for at least 30 times. If the pellet is still not fully resuspended, mildly vortex the tube. We recommend resuspending the RNA pellet immediately after drying.
Pellet is insoluble.	• This may happen due to variations in different VTM samples. If insoluble particles are small enough to pass through a 10 µL pipette tip after thorough resuspension, they should have little to no interference with your downstream analysis. Otherwise, we recommend reducing your VTM sample input and follow the protocol instructions for lower input volume.

FREQUENTLY ASKED QUESTIONS (FAQs)

Can I input a different volume of VTM sample than what is indicated on the kit?

The total input volume must equal to 600 µL. More volume will overload the system. If you have less VTM, you must bring the sample volume up to 600 µL by adding Solution A in step 1.

What can I do if I want to increase the purity of the sample?

You can add another 40% isopropanol wash (i.e. repeat steps 6 and 7) before the 70% ethanol wash to increase the purity of the samples. Note, additional washing steps may reduce RNA recovery / yield.

SYMBOLS

IVD	*In vitro* diagnostics medical device		Manufacturer
REF	Catalogue number		Caution
LOT	Lot number		Consult instructions for use
EC REP	European Authorized Representative		Temperature limitation
Σ	Contains reagents sufficient for <n> sample preps		Upper limit of temperature
	Use by	CE	CE Marking

PRODUCT WARRANTY & SATISFACTION GUARANTEE

We warrant that our goods will meet its specifications stated in this manual. This warranty lasts from the time we deliver the product until either the product expiry or "use by" date. If we do not specify the expiry date, the warranty will last for 6 months from the date we deliver the product.

The product may be used solely in accordance with the protocols provided with the product and this manual and for use with components contained in the kit only. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

Our warranty shall not be effective if we determine, in our sole discretion, that you have altered or misused the goods or have failed to use or store them in accordance with instructions furnished by us. Our sole and exclusive liability and your exclusive remedy with respect to goods proved to our satisfaction (applying analytical methods reasonably selected by us) to be defective or nonconforming shall be the replacement of such goods free of charge.

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Information subject to change without notice.
For updated product information, see www.phasescientific.com

PV20UM2051.PVR.108 | PI-1220200ENG | Rev A | For *in vitro* diagnostics |

Limited License Agreement
Use of this product indicate the agreement of any purchaser or user of the PHASIFY™ VIRAL RNA Extraction Kit to the following terms:

1. The PHASIFY™ VIRAL RNA Extraction Kit may be used solely in accordance with the protocols provided with the product and this manual and for use with components contained in the kit only. PHASE Scientific grants no license under any of its intellectual property to use or incorporate the components of this kit with any components not included in this kit except as described in the PHASIFY™ VIRAL RNA Extraction Kit User Manual and additional protocols available at www.phasescientific.com.
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