PHASiFY™ VIRAL

RNA Extraction Kit

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

USER MANUAL

Product Ref. No. 1220100

For Research Use Only

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Contents

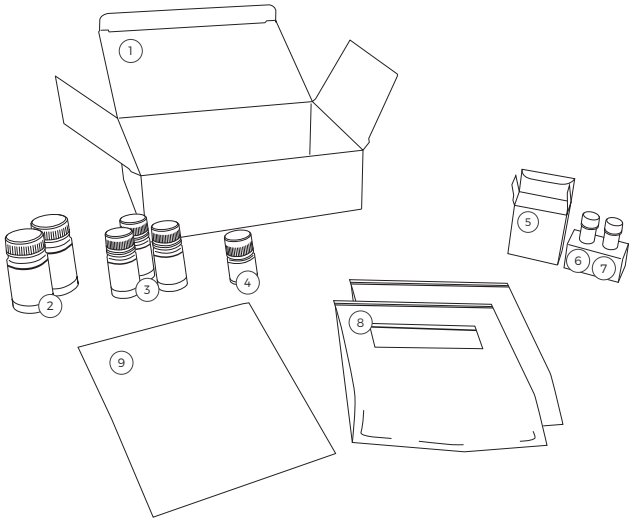
PRODUCT INTRODUCTION

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 Media (VTM), which includes Phosphate-buffered Saline (PBS) and saline. The novel method enables significantly improved sample quality by enabling high sample input (up to 600 µL) and flexible elution volume (as low as 10 µL) to maximize final sample concentration. For research-use only.

Sample Input	Number of Reactions
Up to 600 µL VTM	100

KIT COMPOSITION (VTM SAMPLE INPUT)

Ref. No. 1220100



PHASIFY™ VIRAL RNA Extraction Kit (100 reactions)

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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.

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: Imcomplete 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.

STORAGE CONDITIONS

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.

PRODUCT USE LIMITATIONS

The PHASIFY™ VIRAL RNA Extraction Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease. It is intended for use with human swabs stored in viral transport media (including PBS and saline), and not for any use outside of the product claims.

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.

SAFETY INFORMATION

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.

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.

Ensure that the waste is stored, transferred, transported, and disposed of according to applicable local, state/provincial, and/or national regulations.

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.

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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TECHNICAL ASSISTANCE

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

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

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