PHASiFY™ VIRAL RNA Extraction Kit

100x Reactions (VTM sample input)

Quick Start Protocol

PHASIFY™ VIRAL RNA Extraction Kit Contents

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

Before You Start

Make sure you have prepared the following materials:

Prepare Reagents

- ☐ Solution B1
  Add 875 µL DNase / RNase-free water to a vial of B1 (25 mg) and mix well. Store at 4°C.
- ☐ Master Mix B
  Mix according to the Master Mix B Formulation table found in the kit.
- ☐ Master Mix D
  Mix according to the Master Mix D Formulation table found in the kit.

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

Other Items Required for the Procedure

- ☐ 40% (v/v) isopropanol (molecular-grade)
- ☐ 100% isopropanol (molecular-grade)
- ☐ 70% (v/v) ethanol (molecular-grade)
- ☐ Resuspension buffer
- ☐ Empty microcentrifuge / conical tube
- ☐ Microcentrifuge capable of 4,300 x g
- ☐ Vortex-mixer

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

For research use only.

Download the full user manual and other product resources at www.phasescientific.com/product/viral.

For technical support, contact us at phasify@phasesci.com or at +1 (657) 296 6106 [US]; +(852) 9135 2570 [Hong Kong].

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PI:PV2QS-ENG | PV2QS201-PVR108 | Rev A

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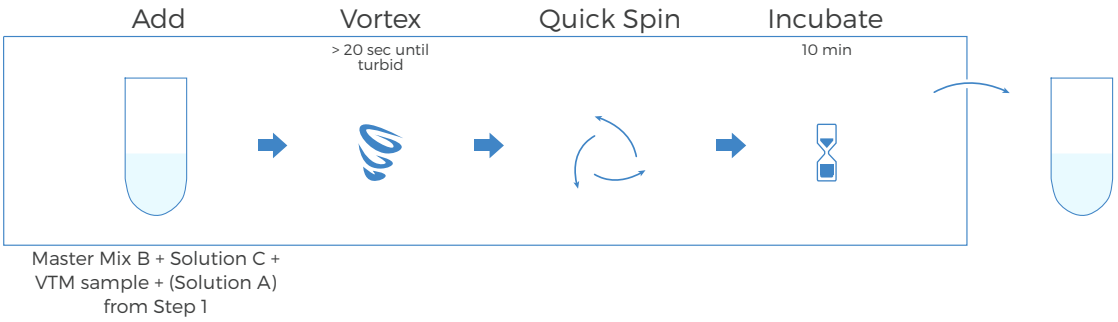
Procedure

Unless specified otherwise, perform the experiment at room temperature.

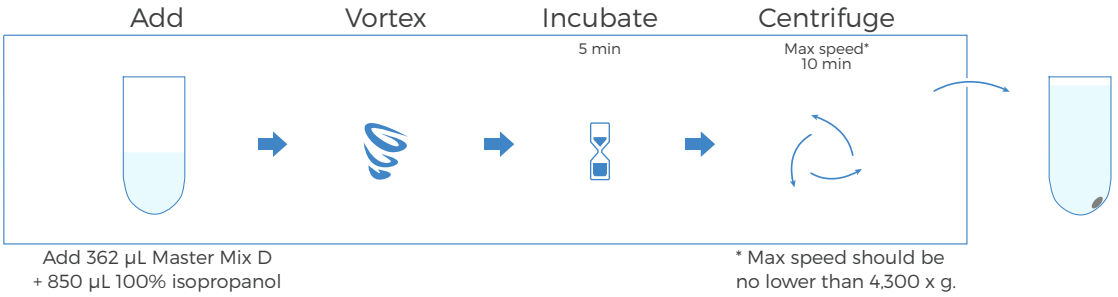
Step 1

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.

Step 2



Step 3



Step 4

- Remove all supernatant and add 1 mL 40% isopropanol
- Centrifuge at max speed for 2 min
- Remove all supernatant and add 1 mL 70% ethanol
- Centrifuge at max speed for 2 min
- Remove all supernatant and dry the pellet for ≥ 10 min until completely dried

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

- Resuspend the pellet in ≥ 10 µL of your buffer of choice. Add buffer directly to the dry pellet. Pipette-mix thoroughly 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.

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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.