Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

Magnetic Viral Nucleic Acid Extraction Kit Instructions

Composition

Magnetic Viral Nucleic Acid Extraction Kit Cat. No.	100 Preps 4012100
Carrier RNA	310 μg×2
Proteinase K	1.2 ml×2
Buffer VLM	60 ml
Magnetic beads	1.2 ml
Buffer WBR（concentrate）	30 ml×2
RNase-free Water	10 ml
Instructions	1

Storage

Carrier RNA Dry Powder and Proteinase K can be shipped at room temperature and stored at -20℃ upon receipt.
Other reagents can be stored at room temperature (0~30℃) and have a validity period of 2 years.

Technical Support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

This product is designed for the extraction of various viral RNA or viral DNA from 200 μl cell-free body fluids (including plasma, serum, urine, CSF, and cell culture supernatant), virus stocks, and virus-infected tissue lysates. The reagents provided in the kit can be prefilled into 2.2 ml 96 deep-well plates, and with the magnetic bead-based nucleic acid automatic extractor, only the sample needs to be added to the well equipped with Buffer VLM, and the instrument can automatically complete a series of processes such as the release, adsorption, washing and elution of viral nucleic acids, and finally the obtained nucleic acids are dissolved in RNase-free Water and can be immediately used for PCR or RT-PCR reactions.

Equipment and Reagents to Be Supplied by User

1. Absolute ethanol and deionized water.
2. RNase-free 1.5 ml centrifuge tubes or 96 deep-well plates (2.2 ml), if you need a prefilled 96 deep-well plate with prefilled reagents and magnetic sleeves, please purchase the prefilled Magnetic Viral Nucleic Acid Extraction Kit (Cat. No. 4012064).
3. RNase-free pipettes and tips (to avoid contamination between samples, use pipette tips with filters).
4. Disposable gloves and protective equipment and tissues.
5. Magnetic stands, water baths, and vortexer or magnetic bead-based automated nucleic acid extractors.

Preparation Before Use

1. Each tube of Proteinase K was added to 1 tube of Carrier RNA dry powder and vortexing until all Carrier RNA was dissolved. Proteinase K with Carrier RNA should be stored at -20℃ for 6 months without affecting the use effect.
2. Add absolute ethanol to Buffer WBR according to the instructions on the label of the reagent bottle and mark it with "Ethanol added" by ticking the box on the label.
3. For manual extraction, set the water bath temperature to 60℃ and incubate the RNase-free water at 60℃.

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Protocol (Automated Extraction)

1. Prefill reagents in 96-well plates as per Steps A~ E:

A. Premix the two solutions as every 1 ml of Buffer VLM add 40 µl Proteinase K with Carrier RNA, then add 500 µl Buffer VLM with Carrier RNA and proteinase K to each well in columns 1 and 7 of a 96 deep-well plate.

B. Add 800 µl Buffer WBR per well in columns 2 and 8 of the 96 deep-well plates.

C. Add 800 µl Buffer WBR per well in columns 3 and 9 of the 96 deep-well plates.

D. Shake the cryovial containing the magnetic beads vigorously to fully suspend the magnetic bead particles in the buffer and add 40 µl magnetic beads and 260 µl deionized water to each well in columns 4 and 10 of the 96 deep-well plate.

E. Add 80 µl RNase-free Water per well in columns 6 and 12 of the 96 deep-well plate.

！ Note: Viral nucleic acid extraction should be performed immediately after the reagent prefilled is completed, otherwise the ethanol in the Buffer WBR may volatilize, resulting in a decrease in the purity of the final extracted nucleic acid.

2. Add 200 µl sample to each of the wells in columns 1 and 7 of the 96 deep-well plate that has been prefilled and place the 96 deep-well plate into the automated nucleic acid extractor.

3. Set up the program in the nucleic acid extractor as follows:

Step	Hole	Amount of Fluid (µL)	Soak (s)	Stirring intensity (level)	Stirring time (s)	Descending magnetism (s)	Bottom magnetism (s)	Magnetism times	Waiting time (s)	Stop Off/On 0/1	Plate1 lyse (℃)	Plate1 elute (℃)	Plate2 lyse (℃)	Plate2 elute (℃)
1	4	300	0	1	0	30	3	1	0	0	0	0	0	0
2	1	700	0	6	600	30	3	2	0	0	70	0	70	0
3	2	800	0	6	180	30	3	1	0	0	0	0	0	0
4	3	800	0	6	180	30	3	1	600	0	0	0	0	0
5	6	80	0	5	180	30	10	2	0	0	0	85	0	85
6	1	700	0	5	5	5	0	0	0	0	0	0	0	0

* The above Protocol is based on Simgen Automatic Nucleic Acid Extractor (Cat. No. Sim-300), if used with other companies' instruments, please adjust the parameters of the program according to the characteristics of the instrument or call 400-0099-857 for technical support.

4. Transfer the viral nucleic acid in columns 6 and 12 into clean centrifuge tubes or seal the 96 deep-well plates directly with sealing film and store at -70℃ for later use.

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Protocol (Manual Extraction)

Samples are prepared before use

A. Plasma, serum, cell-free body fluid, virus sample preservation fluid, virus stock solution, urine specimens fluid, cerebrospinal fluid, herpes fluid, and cell culture supernatants，etc.:

Use 200 µl sample directly to extraction; if the sample volume is less than 200 µl, add PBS solution to 200 µl.

* Extraction of viral nucleic acids using freshly isolated or freeze-thawed samples no more than one time, whenever possible.

B. Throat swab elution, genital tract swab elution, mouthwash:

Transfer 300 µl sample into a 1.5 ml centrifuge tube, centrifuge at 12000 rpm for 5 min, then transfer 200 µl supernatant for extraction of viral nucleic acids.

C. Virus-infected tissue lysate:

weigh 10 mg virus-infected tissue, grind the sample to powder form with liquid nitrogen, add 300 µl PBS solution for suspension, then transfer 200 µl tissue suspension for extraction of viral nucleic acids.

D. Stool

Add 1 ml of normal saline to a 1.5 ml centrifuge tube, take about 200 mg stool with a sterilized toothpick (if the stool is in liquid form, directly transfer 200 µl directly), add to the 1.5 ml centrifuge tube and vortex until the stool are completely dispersed. Centrifuge at 12,000 rpm for 1 min and transfer 200 µl the top supernatant for extraction of viral nucleic acids.

1. **Transfer 20 µl Proteinase K with Carrier RNA into a 1.5 ml centrifuge tube, then add 500 µl Buffer VLM and 200 µl sample.**

* Carrier RNA can effectively improve the recovery efficiency of trace nucleic acids and protect the RNA from RNase.

2. **Suspend the beads in the cryovial thoroughly, then transfer 40 µl magnetic beads into the 1.5 ml centrifuge tube in step 1, vortex to mix well, incubate at 60°C for 10 min. During the incubation, suspend the magnetic beads by vortexer for a few seconds every 2-3 min to promote the full adsorption of nucleic acids to the magnetic beads.**
3. **Place the 1.5 ml centrifuge tube into the magnetic stand, let it stand for a while, wait for all the magnetic beads to adsorb to the wall the centrifuge tube, discard the solution in the centrifuge tube, and retain the magnetic beads on the wall the centrifuge tube.**

* Spin down the residual liquid from the lid of the 1.5 ml tube into the tube before placing it in the magnetic stand.

4. **Add 800 µl Buffer WBR to the centrifuge tube, vortex for 30 sec to mix well. Place the 1.5 ml centrifuge tube into the magnetic stand, let it stand for a while, wait for all the magnetic beads to adsorb to the wall the centrifuge tube, discard the solution in the centrifuge tube, and retain the magnetic beads on the wall the centrifuge tube.**

* Confirm that absolute ethanol has been added to Buffer WBR.
* Spin down the residual liquid from the lid of the 1.5 ml tube into the tube before placing it in the magnetic stand.

5. **Repeat step 4 once.**
6. **Use a 200 µl pipette tip to carefully discard the solution remaining in the tube, retain the beads on the wall the tube, and let the beads dry for 5-10 min at room temperature.**
7. **Add 80 µl RNase-free Water incubated at 60°C to the magnetic beads, close the lid, vortex for several seconds, then incubate at 60°C for 3min.**

* Extending the incubation time to 10 min, during which vortex for a few seconds every 2-3 min, can effectively improve the elution efficiency of viral nucleic acids.

8. **Place the 1.5 ml tube on the magnetic stand until all the beads are adsorbed to the wall the tube. Transfer the viral nucleic acid solution in the supernatant to an RNase-free 1.5 ml centrifuge tube, and the obtained viral nucleic acid can be immediately used for PCR testing or stored below -70°C for later use.**