**VBI Viral RNA Extraction Kit**

***HANDBOOK***

**Further information**

* Safety Data Sheet: www.virongy.com/safety
* VBI Viral RNA Extraction kit Handbook: www.virongy.com/HB-VBIRNA
* Technical Assistance: info@virongy.com

Features

* Spin column-based purification
* Rapid and reliable isolation
* Binding capacity of 20 µg viral RNA per column
* Simple, user-friendly protocol
* High purity RNA for qPCR, sequencing, digestion, cloning, and other applications

Contents

Description1

Intended Use1

Kit Components2

Storage2

Safety Information2

Equipment’s and materials supplied by the user2

Procedure 3

Troubleshooting 3

1. **DESCRIPTION**

VBI Viral RNA extraction kit provides column-based rapid isolation of high-quality RNA from fresh or frozen viral preps with a binding capacity of 20 µg per column. The kit uses well-established technology for RNA samples from volumes and sizes (e.g., 20 µL -200 µL virus samples). The extracted RNA is suitable for use in many downstream applications, including RT-PCR and next generation sequencing.

1. **INTENDED USE**

This product has been manufactured for research use purposes. This product has not been developed for the treatment or diagnosis of a disease on humans or animals.

**THE PRODUCT IS INTENDED FOR USE ONLY BY PROFESSIONALS WHO HAS BEEN TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES.**

*This kit is used for isolation of viral RNA from a variety of RNA viruses, but performance cannot be guaranteed for every RNA virus.*

1. KIT COMPONENTS

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| VBI Viral RNA Extraction Kit | 10 | 25 | 50 | 100 |
| CATALOG no. | VBIRNA-R10 | VBIRNA-R25 | VBIRNA-R50 | VBIRNA-R100 |
| RNA Extraction Buffer | 4 ml | 5.5 ml | 18 ml | 36 ml |
| Washing Buffer | 1.4 ml\* | 3 ml\* | 6 ml\* | 13 ml\* |
| Elution Buffer | 0.5 ml | 1 ml | 2 ml | 4 ml |
| Collection Tubes (2 ml) | 20 | 50 | 100 | 200 |
| VBI Binding Column | 10 | 25 | 50 | 100 |

**\*Add Ethanol as listed on section 4.1 before starting**

1. STORAGE

Upon receiving, the kit components should be stored dry at room temperature (15-25°C) unless otherwise stated. VBI binding columns and buffers can be stored and used until the expiration date on the kit box.

1. SAFETY INFORMATION

Please find the Safety Data Sheet (SDS) associated with this product. It is available online.

|  |  |
| --- | --- |
| Warning | *When combined with bleach and/or other acids, this product may produce hazardous gases as it contains Guanidine Salts.* |

1. EQUIPMENT’S AND MATERIALS

**Equipment needed for viral RNA extraction:**

* -20°C freezer for storage of extracted RNA
* Biological Safety Cabinet, BSL-2 or equivalent to work with potentially infectious samples
* Microcentrifuge with an average RCF (Relative Centrifugal Force) of at least 12,000 x g or equivalent.

**Materials not included**

* Personal Protective Equipment (PPE)
* 1.5 mL microcentrifuge tubes
* Ethanol for molecular biology, 100%

1. PROCEDURE
   1. Before Starting

* Carefully read and understand the protocol.
* Add the specified volume of ethanol (100%) to **Washing Buffer** as listed in table below and check the top of bottle indicating this step has been completed.

|  |  |
| --- | --- |
| Kits | Volume (mL) |
| 10 | 5.6 |
| 25 | 15 |
| 50 | 27 |
| 100 | 53 |

Viral RNA Purification Protocol

1. Thaw virus or virus-like particle on ice.
2. Transfer **350 µL** of thawed virus particle to a 1.5 mL microcentrifuge.
3. Add **350 µL** of **RNA Extraction Buffer** to the tube and mix properly.
4. Transfer the above mix of virus with **RNA Extraction Buffer** onto the **VBI Binding column.**
5. Close the lid and centrifuge at 13,000 rpm for 30 seconds.
6. Discard the flow through.
7. Add **650 µL** of **Washing Buffer** and centrifuge at 13,000 rpm for 30 seconds.
8. Discard the flow through.
9. Close the lid and dry the **VBI Binding column** by spinning at 13,000 rpm for 30 seconds.

*This step will remove all the extra residual liquid remaining in the column.*

1. Discard the flow through tube.
2. Transfer the **VBI Binding column** to a clean collection microcentrifuge tube and add **30 µL** of **Elution Buffer** to the column.
3. Incubate for 1 minute at room temperature.
4. Spin the **VBI Binding column** at 13,000 rpm for 1 minute. The elute contains viral RNA. Discard the **VBI Binding column.**
5. For long term storage place the extracted viral RNA at -80°C.
6. Troubleshooting

This guide provides brief recommendations for potential problems with Viral RNA Extraction results. For additional information and assistance, please contact us at [**info@virongy.com**](mailto:info@virongy.com)**.**

|  |  |
| --- | --- |
| Problem | Recommendation |
| RNA Low yield | Nucleases may have degraded RNA. Follow recommended storage and handling conditions of your sample type  Starting material size is insufficient. Use more materials |
| RNA Degradation | Poor quality of samples. Always use fresh samples or samples frozen at -80°C. Follow recommended storage and handling conditions of your sample type. |
| Poor performance of RNA | Prepare buffers as directed in section 7.1 (***Before Starting***). Ensure that 100% ethanol is added.  Traces of ethanol from Wash Buffer can inhibit downstream reactions. Make sure the ***step 8*** is strictly followed.  Ensure that the reagents used for performing downstream applications are within the specifications and expiration date |