GeneDia

Viral RNA Extraction Kit MANUAL

GENEDIA™ **life Science Co**.

Product # EK0150R

 # EK01100R

 # EK01200R

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Introduction

The **GENEDIA™ Viral RNA Extraction Kit** (spin column) can extract viral RNA from biological fluid samples e.g. plasma, serum, saliva and nasal sample, but not blood and ensures maximum removal of protein and other organic compound impurities. The extracted viral RNA can be directly used for downstream applications such as RT-PCR, qRT-PCR experiment. The amount of purified viral nucleic acid depends on the sample type, the virus load, sample source, storage condition, and age.

Kit Specifications

With the **GENEDIA™ Viral RNA Extraction Kit**, RNA viruses are lysed quickly and efficiently by Lysis Buffer –RVLB- which is a highly concentrated solution of guanidinium salts. Lysis buffer and ethanol create appropriate conditions for binding of nucleic acids to the silica membrane of RNA Virus Spin Columns. Contaminations (potential PCR inhibitors) like salts, metabolites and soluble macromolecular cellular components are removed in simple washing steps with buffers RVWB1 and RVWB2. The nucleic acids can be eluted in RVEB.

Kit Components

Components	Product # EK0150R (50 preps)	Product # EK01100R (100 preps)	Product # EK01200R (200 preps)
RVLB (Lysis)	15 ml	30 ml	60 ml
RVWB1 (Wash 1)	12 ml	22 ml	43 ml
RVWB2 (Wash 2)	7 ml	13 ml	25 ml
RVEB (Elution)	2 ml	4 ml	8 ml
Proteinase K	0.5 ml	1 ml	1 ml *2
Spin Columns	50	100	200
Elution tubes	50	100	200
Product Insert	1	1	1

Storage Conditions

All components of the **GENEDIA™ Viral RNA Extraction Kit** should be stored at room temperature (20-25 °C) and are stable for 1 year. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

Recommended Equipment and Reagents

- 56-65 °C incubator
- Benchtop microcentrifuge
- Sampler in 100 to 1000 Microliter size
- Sampler tips
- 2 ml microcentrifuge tubes

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- Vortex
- Absolute Ethanol
- DNase (if DNA-free RNA is required)

Precautions and Disclaimers

- Prior to using the protocol, Genetic ID recommends that care be taken in homogenizing the sample in a manner that minimizes the risk of inadvertently contaminating the sample. Contamination can occur using improperly cleaned equipment or using poor laboratory practices during homogenization, weighing and labeling of the subsample.
- The Buffer RVLB contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.
- All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})(r)}}$$

Where RCF = required gravitational acceleration (relative centrifugal force in units of g); r = radius of the rotor in cm; and RPM = the number of revolutions per minute required to achieve the necessary g-force.

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.

Notes Prior to Use

• Prepare a working concentration of the **RVWB1** by adding:

- **5 ml** of absolute Ethanol (not provided) to each of the bottles containing **12 ml** of concentrated **RVWB1**. This will give a final volume of 17 ml for **Product # EK0150R**
- **9 ml** of absolute Ethanol (not provided) to the supplied bottle containing **22 ml** concentrated **RVWB1**. This will give a final volume of 31 ml for **Product # EK01100R**
- **18 ml** of absolute Ethanol (not provided) to the supplied bottle containing **43 ml** concentrated **RVWB1**. This will give a final volume of 61 ml for **Product # EK01200R**

The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

• Prepare a working concentration of the **RVWB2** by adding:

- **10 ml** of absolute Ethanol (not provided) to each of the bottles containing **7 ml** of concentrated **RVWB2**. This will give a final volume of 17 ml for **Product # EK0150R**

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- **19 ml** of absolute Ethanol (not provided) to the supplied bottle containing **13 ml** concentrated **RVWB2**. This will give a final volume of 32 ml for **Product # EK01100R**
- **37 ml** of absolute Ethanol (not provided) to the supplied bottle containing **25 ml** concentrated **RVWB2**. This will give a final volume of 62 ml for **Product # EK01200R**

The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

A. Sample preparation

• The steps for preparing the lysate are different depending on the starting material (lysate preparation step). However, the subsequent steps are the same in all cases (binding Step– elute Viral RNA step).

• Please ensure that the correct procedure for preparing the lysate from your starting material is followed, as indicated below:

A.1 Lysate Preparation from Nasal or Throat Swabs

• Body fluid of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with these samples.

1) Add **300 µl of RVLB** & **10 µl Proteinase K** to a microcentrifuge tube (not provided).

(Optional): *DNase Treatment: If DNA-free RNA is required, add the equivalent of 100 units of DNase to the lysate.*

2) Gently brush a sterile, single use Dacron swabs inside the nose or mouth of the subject.
3) Cut the Swab where the nasal or throat cells were collected and place into the microcentrifuge tube containing the Lysis Solution. Close the tube.
4) Vortex gently and incubate at **58 °C** for **15 minutes** and after the lysing process wait for the sample to reach room temperature.
5) Squeeze out the swab and discard it.
6) Proceed to step 7 (Binding Viral RNA to Column).

A.2 Lysate Preparation from free Plasma/Serum

1) Transfer up to **150 µl** of **plasma** or **serum** to a microcentrifuge tube (not provided).
2) Add **200 µl of RVLB** & **10 µl Proteinase K** to plasma or serum.

(Optional): *DNase Treatment: If DNA-free RNA is required, add the equivalent of 100 units of DNase to the lysate.*

3) Vortex gently and incubate at **58 °C** for **15 minutes** and after the lysing process wait for the sample to reach room temperature.
4) Proceed to step 7 (Binding Viral RNA to Column).

A.3 Lysate Preparation from Universal Transport Medium for viruses.

1) Pipette 1ml of sample (transport medium or diluted sputum or saliva) into 1.5 ml tubes (not provided) and centrifuge for **10 minutes** at **11,000 x g** (~ 10,000 RPM).
2) Remove supernatant without dislodging the pellet.
3) Add **300 µl of RVLB** & **10 µl Proteinase** to cell pellet.

(Optional): *DNase Treatment: If DNA-free RNA is required, add the equivalent of 100 units of DNase to the lysate.*

4) Vortex gently and incubate at **58 °C for 15 minutes** and after the lysing process wait for the sample to reach room temperature.
5) Proceed to step 7 (Binding Viral RNA to Column).

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B. Binding Viral RNA to Column

7) Add **400 µl Absolute Ethanol** (Not provided) and mix by vortexing (2 x 5 s).
8) Assemble a Spin column with collection tube.
9) Transfer onto the column and incubate for **2 minutes** at room temperature.
10) Centrifuge at **8,000 x g** (~ 6,000 RPM) for **2 minutes**.
11) Discard the flow through. Reassemble the spin column with its collection tube.

Typically, samples will pass through the columns within ≤ 1 minute (in less than 1 minute). If the entire volume has not passed, spin for an additional minute.

C. Column Wash

1st wash

12) Add **300 µl Buffer RVWB1** to the GENEDIA Viral RNA Spin column.
13) Centrifuge at **11,000 x g** (~ 10,000 RPM) for **1 minutes**.
14) Discard the flow through. Reassemble the spin column with its collection tube.

2nd wash

15) Add **300 µl Buffer RVWB2** to the column and centrifuge for **1 minute** at **11,000 x g** (~ 10,000 RPM).
16) Discard the flowthrough and reassemble the spin column with its collection tube.
17) Spin the column for **2 minutes** in order to thoroughly dry the resin. Discard the collection tube.

D. Elute Viral RNA

18) Place the column into a new Elution tube provided with the kit.
19) Add **40 µl** of **RVEB** (preheated at 60°C) to the column. Incubate the assembly at room temperature for **2 minutes**.
20) Centrifuge for **1 minute** at **12,000 x g** (~12,000 RPM).

***Note**: For an improved yield, elute the sample twice and use after concentration process.*

E. Storage of Viral RNA

The purified Viral RNA may be stored at –20 °C for a few weeks. It is recommended that samples be placed at –70 °C for long term storage.

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Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor Viral RNA Recovery	**Incomplete lysis of cells or tissue**	Ensure that the appropriate amount of Digestion Buffer RVLB with Proteinase K added was used. Increase the incubation time
	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also "Clogged Column" below.
	Low Viral RNA content in cells or tissues used	Different tissues and cells have different Viral RNA contents, thus the expected yield of Viral RNA will vary greatly from these different sources. Please check literature to determine the expected Viral RNA content of your starting material.
Clogged Column	**Clarified lysate was not used for the binding step**	Ensure that after the lysis step the sample is centrifuged if a significant amount of debris is present, and that only the clarified lysate is used in subsequent steps.
RNA does not perform well in downstream applications	**Ethanol carryover**	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications

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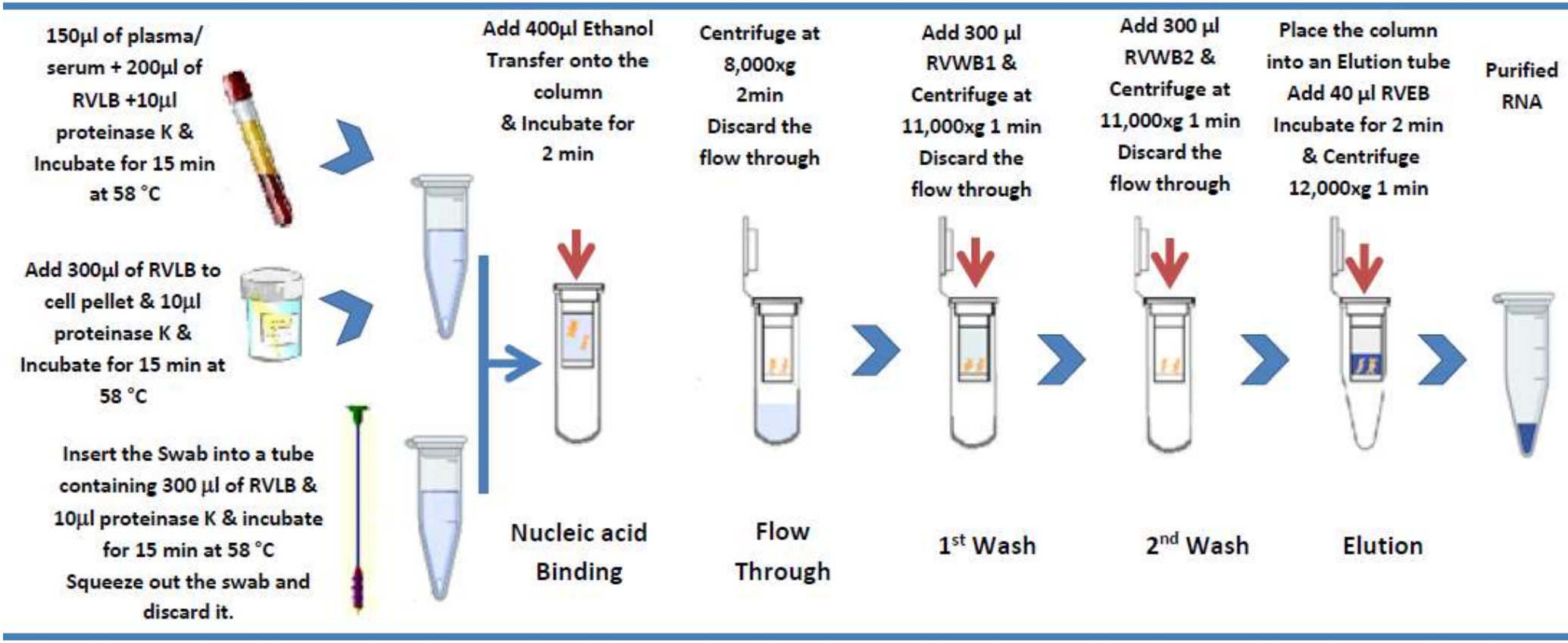
Isolation Method of Viral RNA

Workflow



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