Viral DNA Extraction Kit MANUAL

GENEDIA™ **life Science Co**.

Product # EK0250R

 # EK02100R

 # EK02200R

Unit 1, Ground Floor, No.14 Building, End of the Taghavi Alley, South Bahar St, Kaveh Blvd, ,Tehran, Iran.
E-Mail: genedialifescience@gmail.com
Phone: +98 21 26601149 +98 21 26601150 +98 922 652 5584

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E-Mail: genedialifescience@gmail.com
Phone: +98 21 26601149 +98 21 26601150 +98 922 652 5584

Introduction

The **GENEDIA™ Viral DNA Extraction Kit** (spin column) can extract viral DNA from tissue; an organ, environmental sample, saliva and nasal sample, and ensures maximum removal of protein and other organic compound impurities. The extracted viral DNA can be directly used for downstream applications. The amount of purified viral nucleic acid depends on the sample type, the virus titer, sample source, storage condition, and age.

Kit Specifications

Purification is based on spin column chromatography using **GENEDIA**'s proprietary resin as the separation matrix. The Viral DNA is preferentially purified from other cellular components without the use of phenol or chloroform. First, the serum or plasma or other type of samples are lysed in the presence of chaotropic salts. Next, Absolute Ethanol is then added to the lysate, and the solution is loaded onto a spin-column. GENEDIA's column binds nucleic acids in a manner that depends on ionic concentrations, thus only the Viral DNA will bind to the column while the contaminants will be removed in the flowthrough or retained on the top of the resin. The bound Viral DNA is then washed with the provided Wash Solutions in order to remove any impurities and the purified Viral DNA is eluted with DVEB.

Kit Components

Components	Product # EK0250R (50 preps)	Product # EK02100R (100 preps)	Product # EK02200R (200 preps)
DVLB (Lysis)	15 ml	30 ml	60 ml
DVWB1 (Wash 1)	12 ml	22 ml	43 ml
DVWB2 (Wash 2)	7 ml	13 ml	25 ml
DVEB (Elution)	2.5 ml	5 ml	10ml
Proteinase K	0.5 ml	1 ml	1 ml *2
Spin Columns	50	100	200
Collection Tubes	50	100	200
Elution tubes	50	100	200
Product Insert	1	1	1

Storage Conditions

All components of the **GENEDIA™ Viral DNA 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

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- 2 ml microcentrifuge tubes
- Vortex
- Absolute Ethanol
- RNase A (if RNA-free Viral DNA 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 DVLB 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 **DVWB1** by adding:

- **5 ml** of absolute Ethanol (not provided) to each of the bottles containing **12 ml** of concentrated **DVWB1**. This will give a final volume of 17 ml for **Product # EK0250R**
- **9 ml** of absolute Ethanol (not provided) to the supplied bottle containing **22 ml** concentrated **DVWB1**. This will give a final volume of 31 ml for **Product # EK02100R**
- **18 ml** of absolute Ethanol (not provided) to the supplied bottle containing **43 ml** concentrated **DVWB1**. This will give a final volume of 61 ml for **Product # EK02200R**

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 **DVWB2** by adding:

- **10 ml** of absolute Ethanol (not provided) to each of the bottles containing **7 ml** of concentrated **DVWB2**. This will give a final volume of 17 ml for **Product # EK0250R**
- **19 ml** of absolute Ethanol (not provided) to the supplied bottle containing **13 ml** concentrated **DVWB2**. This will give a final volume of 32 ml for **Product # EK02100R**

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- **37 ml** of absolute Ethanol (not provided) to the supplied bottle containing **25 ml** concentrated **DVWB2**. This will give a final volume of 62 ml for **Product # EK02200R**

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 DNA 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 Tissues

- Viral DNA in tissues is not protected after harvesting until it is disrupted and homogenized. Thus, it is important that the procedure is carried out as quickly as possible, particularly the Cell Lysate Preparation step.
- Fresh or frozen tissues may be used for the procedure. Tissues should be flash-frozen in liquid nitrogen and transferred immediately to a -70°C freezer for long-term storage. Tissues may be stored at -70°C for several months.
- The maximum recommended input of tissue varies depending on the type of tissue being used. The demands on the purification process for tissue samples vary highly between different sample types. Therefore, different input amounts are indicated for individual groups of tissues and additional guidelines are provided in the protocol ensuring that the best possible results are obtained for each tissue type **(APPENDIX A)**.

1) Determine the amount of tissue by weighing. We recommend starting with an input of no more than 10 mg.
2) Transfer the tissue into a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle.
3) Add **300 µl of DVLB** & **15 µl Proteinase K** to the tissue sample and continue to grind until the sample has been homogenized.
4) Incubate for **50 minutes** at **58 °C**.
5) Spin the lysate for **2 minutes** to pellet any cell debris. Transfer the supernatant to another microcentrifuge tube (not provided).
6) Proceed to step 7 (Binding Viral DNA to Column).

A.2 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 DVLB** & **10 µl Proteinase K** to a microcentrifuge tube (not provided).
2) Gently brush a sterile, single use Dacron swab 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 DNA to Column).

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A.3 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 DVLB** & **10 µl Proteinase K** to plasma or serum.
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 DNA to Column).

A.4 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 DVLB** & **10 µl Proteinase K** to cell pellet.
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 DNA to Column).

B. Binding Viral DNA 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 flowthrough. Reassemble the spin column with its collection tube
Typically, samples will pass through the columns within ≤ 1 minute (in less than 1 96 minute). If the entire volume has not passed, spin for an additional minute.

C. Column Wash

1st wash

12) Add **300 µl Buffer DVWB1** to the GENEDIA Viral DNA Spin column.
13) Centrifuge at **11,000 x g** (~ 10,000 RPM) for **1minute**.
14) Discard the flowthrough. Reassemble the spin column with its collection tube.

2nd wash

15) Add **300 µl Buffer DVWB2** 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 DNA

18) Place the column into a fresh Elution tube provided with the kit.
19) Add **50 µl** of **DVEB** (preheated at 60°C) to the column. Incubate the assembly at room temperature for **2 minutes.**
20) Centrifuge for **2 minutes** 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 DNA

The purified Viral DNA 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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Appendix A

Choosing Input Amounts

The table included below shows recommended and maximal input amounts for the various sample types that can be processed with the viral DNA Purification Kit. Additionally, typical yields and DIN values are shown. Using input amounts that exceed the recommended amount will lead to a reduction of yield and purity in those samples. If more starting material is required, splitting the sample and processing on multiple columns is recommended.

SAMPLE TYPE	RECOMMENDED INPUT	AMOUNT TYPICAL YIELD (µg)	DIN	MAXIMUM INPUT AMOUNT
Ear	**10 mg**	18–21	**8.5-9.5**	**10 mg**
Liver	**10 mg**	15–30	**8.5-9.5**	**10 mg**
Kidney	**10 mg**	10–25	**8.5-9.5**	**15 mg**
Spleen	**10 mg**	30–70	**8.5-9.5**	**10 mg**
Heart	**10 mg**	9–10	**8.5-9.5**	**25 mg**
Lung	**10 mg**	14–20	**8.5-9.5**	**15 mg**
Brain	**10 mg**	4–10	**8.5-9.5**	**12 mg**
Muscle	**10 mg**	4–7	**8.5-9.5**	**25 mg**

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Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor Viral DNA Recovery	**Incomplete lysis of cells or tissue**	Ensure that the appropriate amount of Digestion Buffer DVLB with added Proteinase K 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 DNA content in cells or tissues used	Different tissues and cells have different Viral DNA contents; thus, the expected yield of Viral DNA will vary greatly from these different sources. Please check literature to determine the expected Viral DNA 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 number of debris is present, and that only the clarified lysate is used in subsequent steps.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitate formation that can clog the columns.
DNA 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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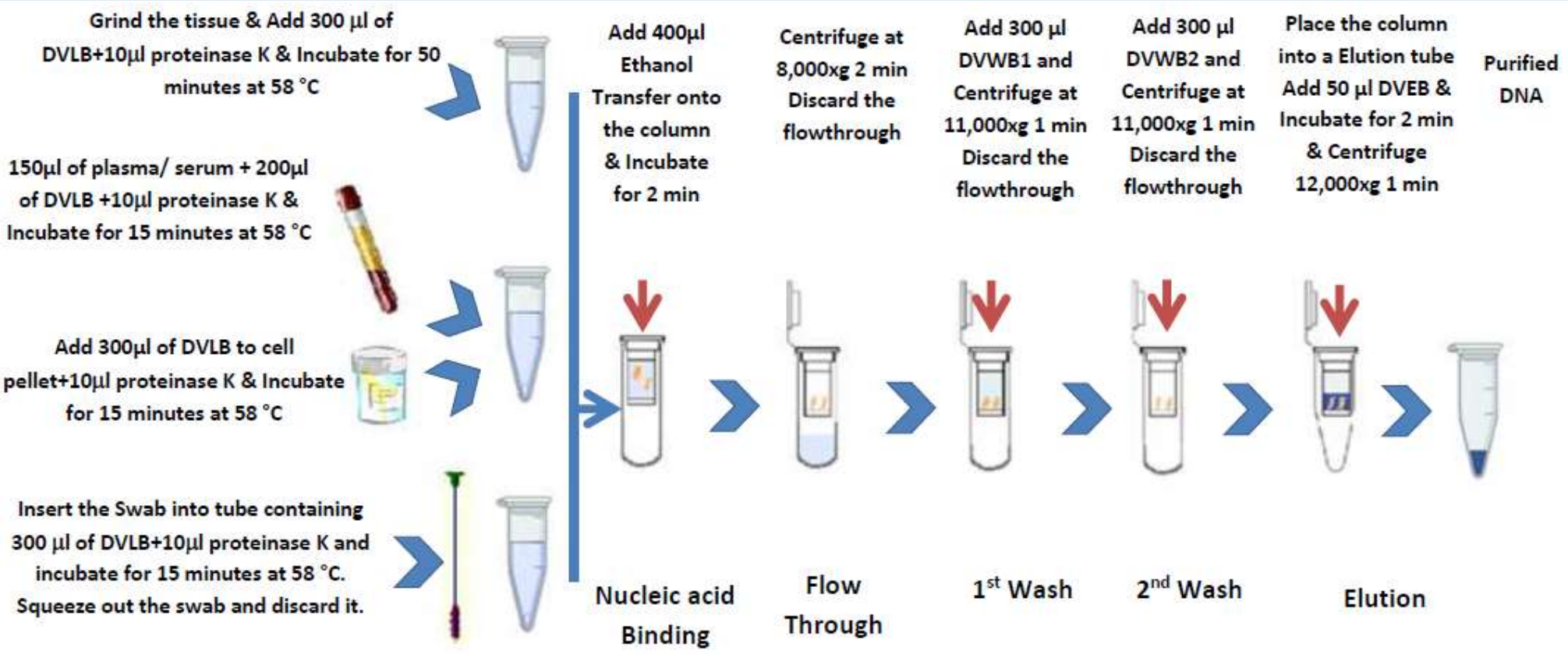
Isolation Method of Viral DNA

Workflow



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