ePure Viral Nucleic Acid Extraction Kit

Instructions for Use (Handbook)

REF E2003

Version: 1.1

48

For *in vitro* diagnostic use

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Read and follow these Instructions for Use prior to using this product. The latest revision of this document can be found at www.ecolidx.com

Rev 02/22

Contents

Intended Use

ePure Viral Nucleic Acid Extraction Kit contains all required reagents and consumables for the rapid automated purification of high quality Viral DNA and RNA from serum, plasma or cell-free fluids of 100-400 µL using proven magnetic particle separation technology. The isolated Viral DNA/RNA of exceptional purity is suitable for PCR and RT-PCR, Genotyping or Sequencing (NGS) assays.

The product is intended to be used by professional users, such as technicians and physicians who are trained in molecular biology techniques.

Introduction

Product Name	ePure Viral Nucleic Acid Extraction Kit
Catalogue Number	E2003
Product Overview	ePure Viral Nucleic Acid Extraction Kit is intended to be used in combination with the ePure series instruments. eEPure Viral Nucleic Acid Extraction Kit provides reagents for fully automated and simultaneous purification of viral nucleic acids from human biological specimens such as serum, plasma, and other cell-free fluids. However, performance characteristics for every biological specimen types have not been established and must be validated by the user. The unique magnetic beads technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities. Purified nucleic acids are ready for direct use in downstream applications.
Applicable Instrument Model	ePure instrument
Display Protocol Name on The Instrument	2003 VIRAL 2003 VIRAL RAPID
Processing Time	ePure 45-51 minutes ePure 17-20 minut (Rapid protocol)

Kit Contents and Storage

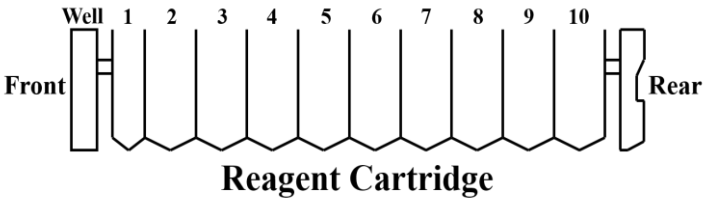
Shipping and Storage	The Kit is shipped at room temperature. Upon receipt, store the Kit at room temperature. All Kit components are stable when stored properly until the expiration date shown on the kit box.
Kit Content	The components supplied in the Kit are listed below. Sufficient reagents are supplied to perform 48 purifications.

Contents	Amount
1 Reagent Cartridge	48 pcs (6x8)
2 Reaction Chamber	48 pcs (6x8)
3 Tip Holder	48 pcs (6x8)
4 Piercing Pin	50 pcs
5 Filter tip	50 pcs
6 Sample Tube (2 mL)	50 pcs
7 Elution Tube (1.5 mL)	50 pcs
RNA Carrier (1 mg)	1 pc
Barcode sticker (on request)	50 pcs
Selection guide (option)	1 pcs

Reagent Cartridge Contents

Each Reagent Cartridge has 10 positions with 10 sealed well. Positions 1-10 contain wells filled reagents for this protocol

Reagent	Well No.
Proteinase K Solution	1
Lysis Buffer 4	2
Binding Buffer 1	3
Magnetic Bead Solution	4
Washing Buffer 2	5
Washing Buffer A	6
Washing Buffer B	7
RNase-free water	8
RNase-free water	9
Empty	10



Reagent Cartridge

Materials Required Not Provided

The following general laboratory equipment and consumables are required to perform the Kit. All laboratory equipment should be installed, calibrated, operated, and maintained according to the manufacturer's recommendations. The following tables display required and special equipment along with the list of consumables.

Item
ePure instrument
1.5 or 2.0 mL micro-centrifuge tubes
Pipettes and filter tips
Phosphate-buffered saline (PBS, may be required for diluting samples)
Optional: Plastic consumables, DNase-free RNase A (to minimize RNA content)

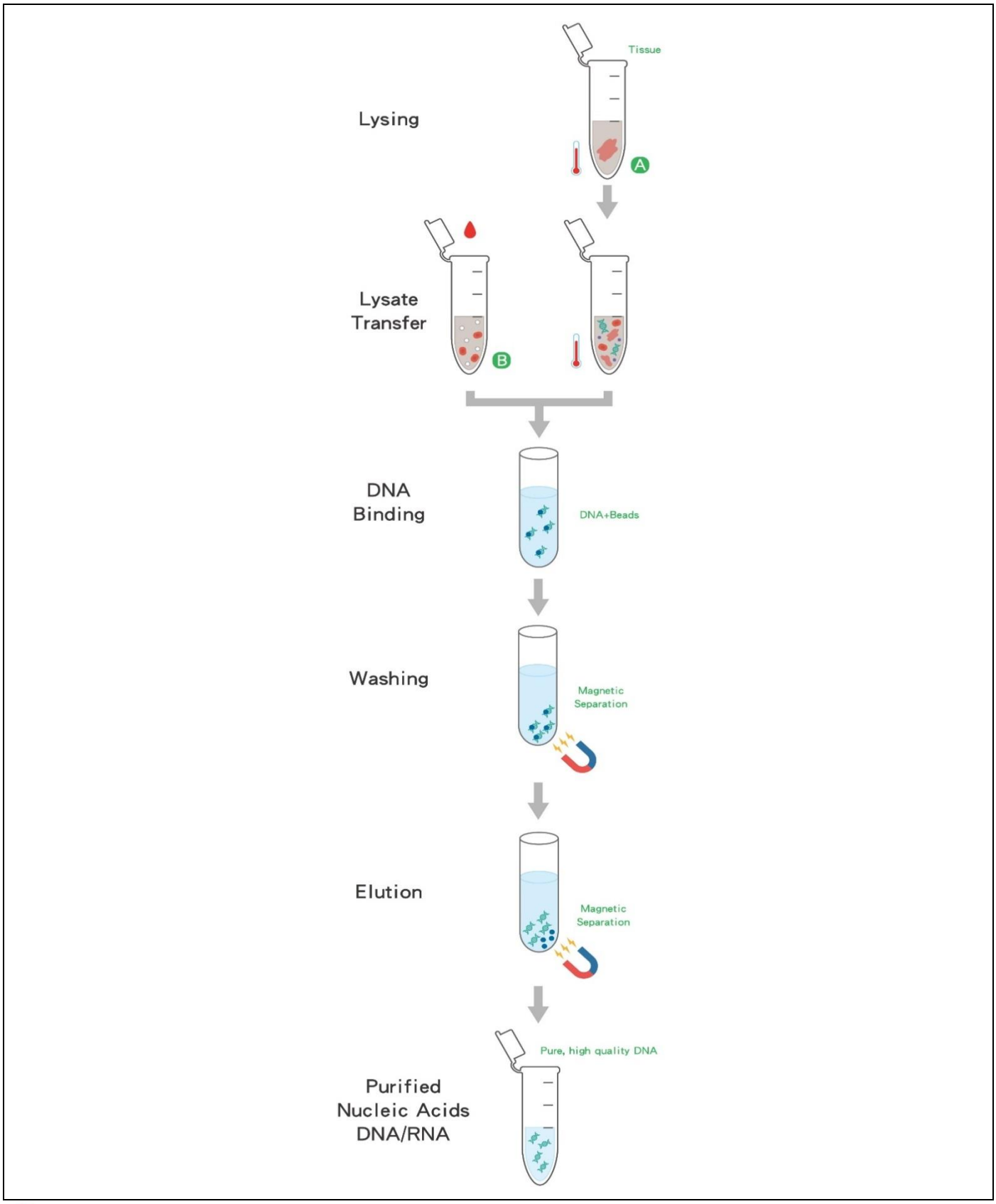
Warnings and Precautions

For *in vitro* diagnostic use only. Read all the instructions carefully before using the kit. Use of this product should be limited to trained personnel in the techniques of DNA purification. Strict compliance with the user manual is required for optimal results. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use a kit after its expiration date.

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (MSDSs, download at www.ecolidx.com).

CAUTION: DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Purification Principle



A Transfer sample to extraction directly.

Perform certain pretreatment process before extraction.

Before Starting

Preparation of sample materials

The purification procedure is optimized for the use of 100-400 µl serum, plasma*, CSF, pretreated urine or other cell-free body fluid samples.

	Serum	a. Add an appropriate volume of RNA carrier into each sample tube. b. Dispense 100-400 µl of sample into each sample tube. c. If the sample volume is lower than described, please complete with an appropriate volume of 1X PBS.
	Plasma	a. Add an appropriate volume of RNA carrier into each sample tube. b. Dispense 100-400 µl of sample into each sample tube. c. If the sample volume is lower than described, please complete with an appropriate volume of 1X PBS.
	Cerebrospinal fluid (CSF)	a. Add an appropriate volume of RNA carrier into each sample tube. b. Dispense 100-400 µl of sample into each sample tube. c. If the sample volume is lower than described, please complete with an appropriate volume of 1X PBS.
	Urine	a. Centrifuge sample at 20,000 x *g* for 10 minutes to concentrate virus into a pellet. b. Discard supernatant and re-suspend the pellet in 220 µl 1X PBS. c. Transfer 200 µl concentrated sample into sample tube.
	Cell-free body fluid(s)	a. If sample contain less than 1 x 10^3 leukocytes / µl, concentrate the blood cells by centrifuge at 1660 x *g* for 15 minutes at 4°C. b. Discard excess of supernatant except 100-500 µl and resuspend. c. Dispense 100-400 µl leukocyte concentrate (no more than 5 x 10^6 cells) into each sample tube.
	Swab Samples	a. Collect swab samples (e.g., eye, nasal, pharyngeal, or other swabs) in liquid transport media or 1 ml PBS containing a common fungicide. b. Incubate for 30 minutes at room temperature. c. Dispense 100-400 µl of sample into each sample tube.

The suggested starting material and elution volume ranged for each nucleic acid extraction

Sample type	Starting material per sample
Serum	100-400 µl
Plasma	
Cerebrospinal fluid (CSF)	
Pretreated Urine	100-400 µl large volume liquid sample pretreatment
Cell-free body fluids	100-400 µl

Important

*Plasma must be prepared from fresh or frozen blood samples collected in tubes which contains common anti-coagulants like EDTA and citrate. (Heparin has inhibitory effect on nucleic acid amplification reaction)

RNA Carrier has two roles in the purification process. First, it enhances the binding of viral nucleic acids to the silica surface of magnetic particles, especially when there are few target molecules in the sample. Second, in rare cases, RNase will not be denatured by chaotropic salts and detergents in the lysis buffer when RNA carrier is present. If RNA carrier is not added to the reaction, recovery of DNA or RNA may be reduced.

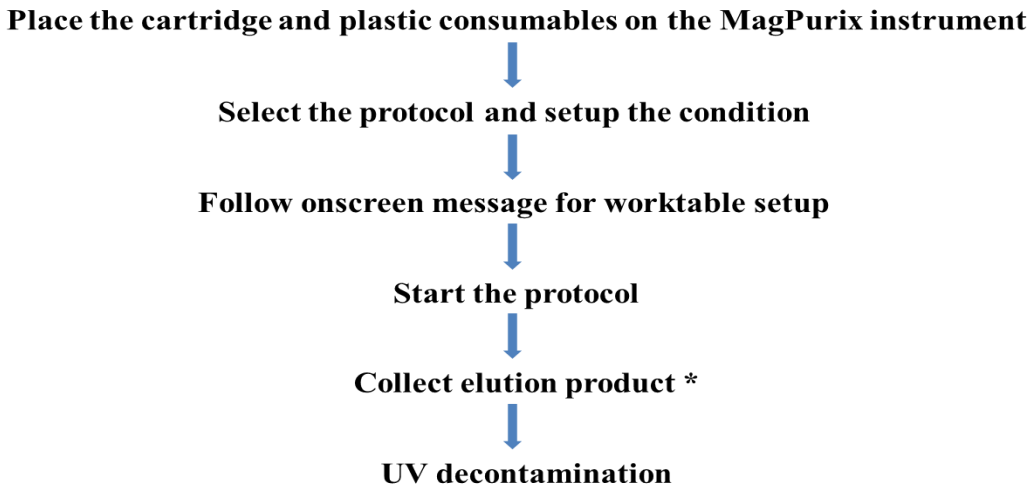
Use fresh sample (stored at 2-8°C for up to 6 hours) for extraction is recommended. Total nucleic acid yield and quality will decrease with time or after multiple freezing – thawing repetitions. For longer storage time, samples should be frozen at -20°C or lower and avoid freezing-thawing repetitions. Thaw samples at room temperature (15-25°C) and process the sample immediately after equilibration to room temperature. **Do not** refreeze sample after thawing. If precipitation is visible in sample, centrifuge at 6,800 x g for 3 minutes and transfer supernatant to a new tube without disturbing the precipitate, and immediately start the purification procedure.
*For large volume liquid samples with low or unknown viral content, e.g. urine or other, follow the “Urine” preparation concentrate procedure.

Preparation of RNA Carrier

RNA Carrier	a.	Gently spin the RNA Carrier tube before opening it.
	b.	Add 1.0 ml RNase-free water to lyophilized RNA Carrier (supplied) and mix by vortex.
	c.	Store RNA Carrier at 4°C (short-term, up to 1 month) or -20°C (long-term, aliquots before freezing). Avoid freeze-thawing repetitions more than 3 times.
	d.	Before extraction, add 5 µl RNA carrier (for 100 µl sample), 10 µl (for 200

µl sample) or 20 µl (for 400 µl sample) into Sample Tube.

Procedure for Viral Nucleic Acids purification



* Output the bench record (option)

2 protocols for this kit are available:

2003 VIRAL – standard protocol for Viral DNA/RNA extraction

2003 VIRAL RAPID – shortened extraction protocol (e.g. SARS-CoV-2)

Purification Protocol on ePure

1	Turn on the Instrument	a.	Turn ON the power switch - and wait for the screen to turn ON
		b.	Login and show the Home Page.
2	Load new Consumable(s) and Cartridge(s)	a.	Open the door and remove the sample rack from the instrument.
		b.	Open the Tip-Holder Lid.
		c.	Load 1 Reagent Cartridge, and all plastic disposables (2 Reaction Chamber, 3 Tip Holder, 4 Piercing Pins, 5 Filtered Tips and other components if present in the kit intended to use).
		d.	Close the Tip-Holder Lid.
		e.	Paste the Barcode sticker on the Elution Tubes (optional).
		f.	Place 6 Sample Tubes and 7 Elution Tubes into the Sample Rack.
3	Transfer samples into instrument	a.	Transfer appropriate volume of sample into sample tubes on sample rack.
		b.	Put back the sample rack into the instrument and Close the door.
4	Program Set up	a.	Select the appropriate protocol program on the instrument. Press **NEXT**.
		b.	Select an appropriate Sample Volume / Elution Volume and press **NEXT**.

		c.	Press the number button to select the right Sample Numbers.
		d.	Scan / Edit each primary Sample ID directly. After finished, Press **NEXT**.
		e.	Scan / Edit each Elution Tube ID directly. After finished, Press **NEXT**.
		f.	Scan Reagent Cartridge Barcode. Press **NEXT**. *If the cartridge expired, the next step cannot be performed.
		g.	Follow the instructions on screen to double-check the operating steps being completed before running the program. Press **NEXT**.
5	Start Extraction	a.	Check "**PROGRAM CONFIRMATION**" on screen.
		b.	Press "**START**" to start the experiment. Instrument will run the protocol automatically until whole process is completed.
		a.	At the end of the run (approximately 45-51 minutes, Rapid 19 minutes), instrument alarms briefly and the screen indicates "**PROGRAM FINISH**".
		b.	If you want to perform the same protocol, press "**RERUN**" to perform the same experiment. If you do not re-run the experiment, press the function button "**HOME**" to exit the experiment mode.
6	Collect the Elution tubes	a.	Open the instrument door.
		b.	Collect the elution tubes containing the purified nucleic acids.
		c.	The purified nucleic acids are ready for immediate use. Store the purified nucleic acids at 4°C (short-term, less than 10 days) or aliquot and store at -70°C (long-term) before performing downstream analysis.
		d.	Discard the used cartridges, all plastic consumables into biohazard waste. *Do not reuse the cartridges.
		e.	If you do not continue to use the instrument, return the sample rack back into the instrument, close the instrument door, and press the "**POWER**" function button to enter sleep mode. If the instrument will not be used for a long time, turn off the power switch.

Troubleshooting

This table is helpful for solving common problems. If you need other technical support, please contact ecoli@ecolidx.com or contact your distributor.

Problem	Possible Cause	Comments and suggestions
Poor DNA quality or yield	Deterioration or contamination of reagents.	Please ensure that the kit reagents are still in the effective using period before use. Discard any kit reagent that shows discoloration or evidence of microbial contamination.
	Kit stored under non-optimal conditions	Store kit at 15-25°C at all time after arrival. If either reagent or buffer precipitate upon shipping in cold weather or during long-term storage, dissolve precipitates by gently warming and stirring solution. Please do not freeze the Reagent Cartridges.
	Insufficient sample input	DNA yield depends on the sample type and the number of nucleated cells in the sample. Please proportionally adjust the total input amount of sample to increase the DNA yield.
	Too much of elution buffer was used	The elution volume can be reduced proportionally.
	The eluate of final product(s) is not enough.	Please collect issue information and provide it to your Support Representative / Technical Support as soon as possible.
Clogging issue	Too much sample material was used.	Decrease the input amount of sample material or dilute your sample.
No results in downstream analysis	No signal / The PCR was inhibited.	Using appropriate controls for analysis. Check the positive control, negative control, water (NTC) and internal control to clarify the possible causes.
Instrument malfunction / abnormal sound	Abnormal consumables: 1. Deformed filter tip 2. Deformed reaction chamber 3. Deformed Tip holder	Please replace the batch with normal consumables.
	Abnormal action of instrument: 1. Inaccurate correction value 2. Spare parts or components damaged	Please collect issue information (videos and pictures) and provide it to your Support Representative / Technical Support as soon as possible to calibrate or replace any other

		damaged or worn parts.

Related Products

Contact Ecoli Dx or your sales representative for a complete range of nucleic acid extraction kits from another type of starting material. Following kits are available.

Product Name	Cat. no.
ePure Blood DNA Extraction kit	E2001
ePure Blood DNA Extraction kit 1200	E2002
ePure Viral Nucleic Acid Extraction Kit	E2003
ePure Tissue DNA Extraction Kit	E2004
ePure Bacterial DNA Extraction Kit	E2006
ePure HPV DNA Extraction Kit	E2007
ePure TB DNA Extraction Kit	E2008
ePure FFPE DNA Extraction Kit	E2009
ePure Forensic DNA Extraction Kit	E2010
ePure Viral Pathogen DNA Extraction Kit B	E2012
ePure Plant DNA Extraction Kit	E2014
ePure Total RNA Extraction Kit	E2015
ePure cfDNA Extraction Kit Plus	E2024
ePure cfDNA Extraction Kit LV	E2025

Limited Product Warranty

Ecoli Dx is committed to provide customers with high-quality products and services. Our goal is to ensure that every customer is 100 % satisfied with our products and services. If you have any question or concerns, contact our Technical Support Representatives.

Ecoli Dx guarantees the performance of all products according to the specifications stated in our product literature. The purchaser / user must determine the suitability of the product for his particular use. We reserve the right to change, alter, or modify any product to enhance its performance and design.

No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored and used in accordance with instructions.

Revision History

Version	Date	Description
1.0	15.11.2021	New document
1.1	10.02.2022	New protocol update, new sample type (swabs)