ePure Total RNA Extraction Kit

Instructions for Use (Handbook)

REF E2015

Version: 1.0

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

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Intended Use

The ePure Total RNA Extraction Kit provides a complete set of reagents and consumables for the automated purification of total RNA from mammalian whole blood, Peripheral Blood Mononucleated Cells (PBMCs), animal tissues, cultured cells, plant tissue and yeast with ePure system.

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 Total RNA Extraction Kit
Catalogue Number	E2015
Product Overview	The ePure Total RNA Extraction Kit is designed to extract total RNA from mammalian whole blood, Peripheral Blood Mononucleated Cells (PBMCs), animal tissues, cultured cells, plant tissue and yeast. The kit uses unique magnetic technology and in combination with ePure automatic instrument, superior product quality, consistent and high product yield and reproducible results are achieved. The final product is suitable for a wide range of diagnostic and research applications, such as sequencing, genotyping, qPCR, ddPCR and NGS assays.
Display Protocol Name on The Instrument	2015 TOTAL RNA
Processing Time	35-40 minutes

Kit Contents and Storage

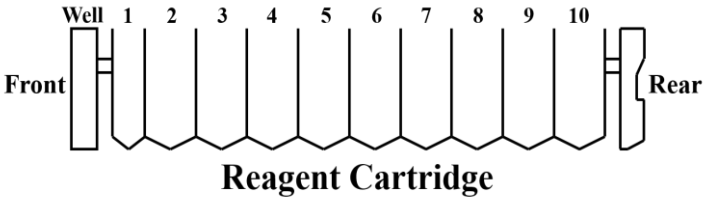
Shipping and Storage	The Kit is shipped at room temperature. Upon receipt, store the Kit at room temperature. All Kit components are stable when stored properly until the expiration date shown on the kit box.
Kit Content	The components supplied in the Kit are listed below. Sufficient reagents are supplied 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
Filter Column	50 pcs
Collection Tube	50 pcs
RLA Buffer (25 mL)	1 pc
RLB Buffer (25 mL)	1 pc
Barcode sticker (on request)	50 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.
Empty	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, RNase-free DNase (to minimize DNA 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).

- Do not use the kit if any consumables are deformed or the cartridge is damaged, or if the conditions of transport and storage according to the instructions for use have not been kept.
- Failure to observe the operating conditions may affect the functions of the kit and the results obtained may not be valid.
- Do not eat, drink, smoke, use cosmetics or handle contact lenses in a laboratory.
- Dispose of all samples and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in a biological box in accordance with appropriate biosecurity procedures.
- Clean and disinfect any spilled samples or reagents with a disinfectant, such as 0.5% sodium

hypochlorite or other suitable disinfectant.

- Avoid contact of samples and reagents with skin, eyes and mucous membranes. In case of contact with these solutions, immediately rinse the affected area with water and, if necessary, disinfect or seek medical attention.
- Danger of explosion and ignition if transport, operation and storage conditions are observed.
- The isolation kit is for single use only on ePure automated extractor for a total sample count of 48. Use the kit only for its intended purpose.
- Any serious adverse event that has occurred in connection with the use of the kit must be reported to the EcoliDx manufacturer and reported in writing to the competent authority of the Member State in which the Instrument is used.
- In the event of a malfunction of the kit or deterioration of its function, which may endanger its functionality, the kit must be discontinued and the manufacturer must be contacted immediately.

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

Quality control

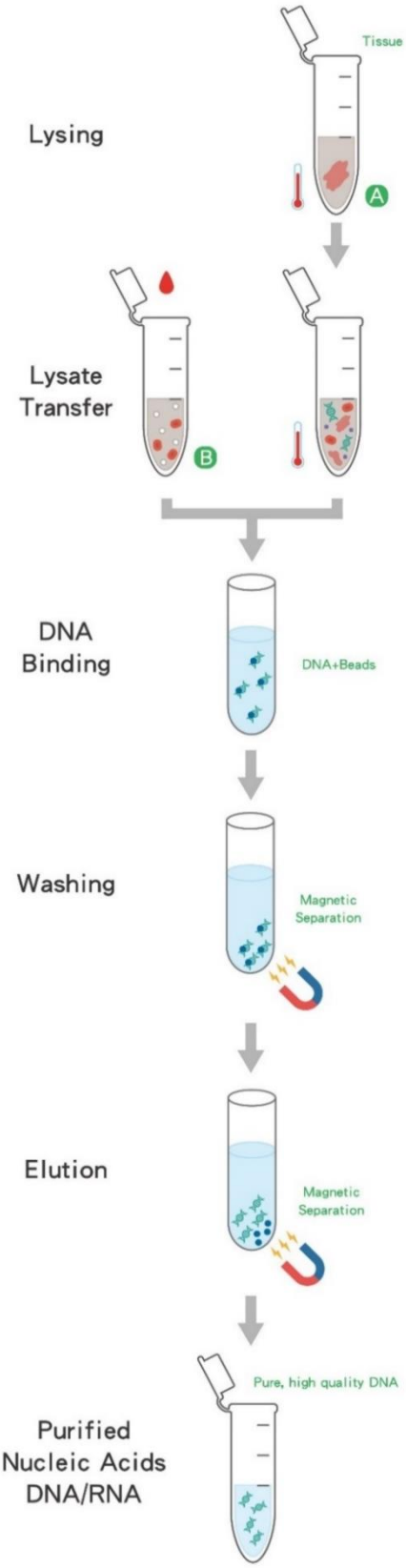
In accordance with the ISO certified EcoliDx quality management system, each kit is tested according to predetermined specifications to ensure consistent product quality.

The following technical standards were also used and complied with for conformity assessment:

ČSN EN ISO 13485 Medical devices - Quality management system - Requirements for regulatory purposes

ČSN EN ISO 14971 Medical devices - Application of risk management to medical devices

Purification Principle



A Transfer sample to extraction directly.

B Perform certain pretreatment process before extraction.

Before Starting

Preparation of sample materials

The purification procedure is optimized for the use appropriate samples as below table		
Mammalian Whole Blood	a.	Fresh prepare 1x RBC lysis buffer.
	b.	Add two part ice-cold RBC lysis buffer to one part blood sample.
	c.	Inverting 3-5 times, incubate on ice for 10-15 minutes.
	d.	Centrifuge at 1.000 x *g*, 10 minutes, 4 °C.
	e.	Remove supernatant.
	f.	Re-suspend the pellet with 220 µl 4°C RL lysis buffer.
	g.	Take 200 µl to sample tube.
Peripheral Blood Mononucleated Cells (PBMCs)	a.	Re-suspend PBMCs with 220 µl 4°C RL lysis buffer.
	b.	Vortex mixing for 10 seconds.
	c.	Take 200 µl to sample tube.
Animal Tissue	a.	Add 220-440 µl 4°C RL lysis buffer to tissue; make sure the sample if completely immersed in buffer. * If the tissue cannot be completely dissolved, a larger amount (up to 440 µl) than the recommended 4°C RL lysis buffer / proteinase K mixture is required.
	b.	Homogenized tissue by homogenizer.
	c.	Spin down the lysate.
	d.	Pre-filter the digested tissue lysate using a filter column to remove residual debris and mucus.
	e.	Centrifuge at 1.000 x *g*, for 5 minutes on 4°C.
	f.	Transfer 200-400 µl to sample tube.
Suspension Culture	a.	Harvest cell culture.
	b.	Centrifuge at 1.000 x *g*, 5 minutes on 4°C.
	c.	Remove supernatant completely.
	d.	Re-suspend cell pellet with 220 µl 4°C RL lysis buffer.
	e.	Vortex mixing for 10 seconds.
	f.	Take 200 µl to sample tube.
Monolayer Culture	Method 1	
	a.	Trypsinize the cells.
	b.	Harvest the cell in PBS.
	c.	Centrifuge at 300 x *g*, 5 minutes on 4°C.
	d.	Remove the supernatant.
	e.	Re-suspend the pellet with 220 µl 4°C RL lysis buffer.
	f.	Vortex mixing for 10 seconds.

	g. Take 200 µl to sample tube. Method 2 a. Scrape the cells with 220-440 µl 4°C RL lysis buffer. b. Vortex mixing for 10 seconds. c. Take 200-400 µl to sample tube.
Plant tissue/ Yeast	a. Add 220-440 µl 4°C RL lysis buffer to sample, make sure the sample if complete immersed in buffer. b. Homogenized tissue by homogenizer. c. Pre-filter the digested lysate using a filter column to remove residual debris. d. Centrifuge at 1.000 x *g*, for 5 minutes on 4°C. e. Transfer 200-400 µl to sample tube.
DNA-free RNA extraction	a. After total RNA program extraction. b. Add 2 µl DNase in the eluate. c. Incubate at 37°C, 10 minutes. d. Transfer the mixture to a new sample tube. e. Proceeding “Total RNA” protocol to start extraction.

Note:

If performing DNA-free protocol, Prepare DNase before extraction. Place 10 µl DNase in the first elute product.

Wear clean glove, use RNase-free filter tip, and keep work area, pipettes and reagents free of virus, bacteria and Nuclease contamination. Using RNase Zap® to clean the surface of bench, equipment and pipettes is one of the easiest way to remove the RNase contaminations of work area.

Using RNA stabilized reagent (e.g., RNA*later*®) to treat sample is one of the best way to protect the RNA if the sample cannot be processing in a RNase-free working area.

Two RL lysis buffers are supplied in the kit for treating different tissue types. User could try both lysis buffers to get the optimized extraction results.

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

Reagent	**Description**	**Preparation**
β-Mercaptoethanol (β-ME)	β-ME reduce disulfide bonds and irreversibly denature the RNase and eliminate RNase released during cell lysis.	Add 10 µl β-ME per 1 ml RL lysis buffer. It can be stored at 4°C for 4 months, at room temperature for 1 month. NOTE: Dispense the β-ME in a fume hood and wear appropriate protective clothing.

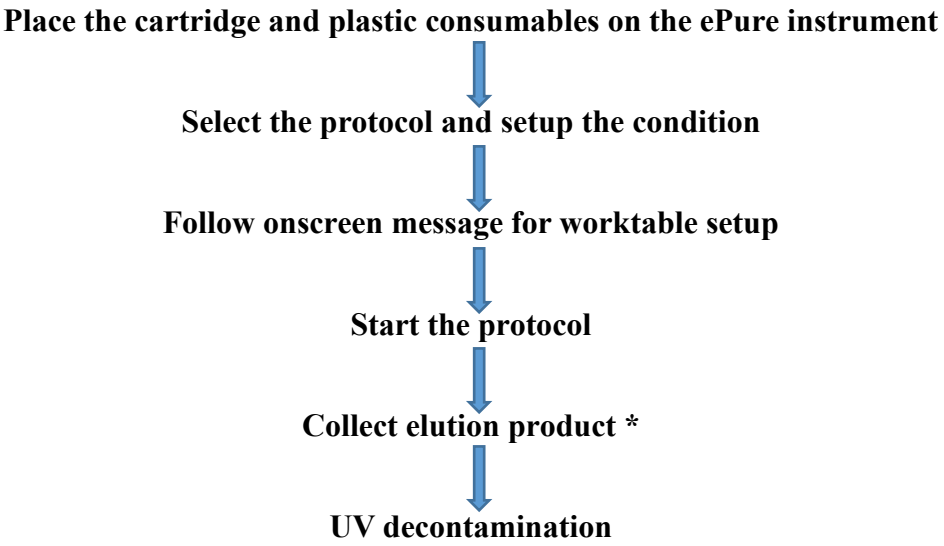
Red blood cells lysis buffer (RBC lysis buffer)	Lyse Erythrocyte from whole blood (Erythrocyte (RBC) lysis procedure)	10x RBC lysis buffer (100 ml) 8.29 g NH4Cl (1.5 M) 1 g KHCO3 (100 mM) 0.0372 g Na2EDTA (10 mM) Adjust pH7.2-7.4 by HCl 0.2 mm filtered, store for 6 months at 4 °C Dilute 10 times fresh before use.
DNase	To eliminate DNA contamination	Novagen RNase-free DNase I (69182-3CN)
10x DNase buffer	To eliminate DNA contamination	0.5 M Tris-HCl 25 mM MgCl2 5 mM CaCl2

See the below table for the suggested starting material and elution volume ranged for each nucleic acid extraction

Sample type	Starting material per sample	Elution Volume
Mammalian Whole Blood	100-400 µl (WBC number is about 1 x 10^6 cells / µl) NOTE: Blood cells needs to perform manual RBC lysis procedure before extraction.	50-200 µl
PBMCs (Peripheral Blood Mononucleated Cells)	200 µl Lysate* * Suspend up to 50 µl sample in 200 µl RL lysis buffer.	
Animal Tissue	200-400 µl / 10 - 40 mg	
Cultured Cell	200-400 µl / up to 5 x 10^6 cells	
Plant tissue	200-400 µl / up to 100 mg	
Yeast	200-400 µl / up to 100 mg	

Isolation procedure using the ePure

Workflow of ePure operation



* Output the bench record (option)

Note: Perform all steps at room temperature (20-25°C) unless otherwise notified.

Purification Protocol- MagPurix® EVO series

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.
		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 program automatically until whole process is completed. c. At the end of the run (approximately 35-40 minutes), instrument alarms briefly and the screen indicates "**PROGRAM FINISH**". d. If you do not re-run the experiment, press the function button "**HOME**" to exist 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.

Storage of isolated RNA

Purified RNA can be stored at -15 ° C to -30 ° C or -65 ° C to -90 ° C in a RNase-free water.

Troubleshooting

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

Problem	Possible Cause	Comments and suggestions
Poor RNA 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	RNA 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 RNA 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

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 CFC DNA Extraction Kit	E2017
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 Jul. 2022	New document release