ePure Forensic DNA Extraction kit

Instructions for Use (Handbook)

REF E2010

Version: 1.0

Σ 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

Contents

Intended Use

ePure Forensic DNA Extraction Kit provides reagents for fully automated and simultaneous purification of DNA from human whole blood, dried blood, hair root, tissues, saliva and other forensic samples, 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 Forensic DNA Extraction Kit
Catalogue Number	E2010
Product Overview	The ePure Forensic DNA Extraction Kit is designed to extract of DNA from human whole blood, dried blood, hair root, tissues, saliva and other forensic samples. 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 such as sequencing, genotyping, qPCR, ddPCR and NGS assays.
Applicable Instrument Model	ePure
Display Protocol Name on The Instrument	2010 FORENSIC DNA
Processing Time	ePure: 45 minut

Kit Contents and Storage

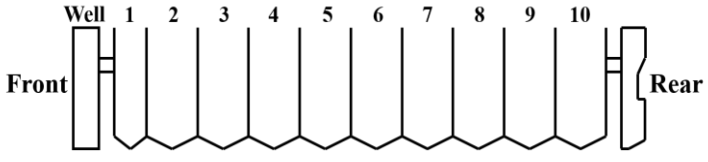
Shipping and Storage	The Kit is shipped at room temperature. Upon receipt, store the Kit at room temperature. All Kit components are stable when stored properly until the expiration date shown on the kit box.
Kit Content	The components supplied in the Kit are listed below. Sufficient reagents are supplied 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
Proteinase K, 10 mg / mL (1 mL)	1 pc
BL2 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 3	2
Binding Buffer 1	3
Magnetic Bead Solution	4
Washing Buffer 1B	5
Washing Buffer A	6
Washing Buffer B	7
Elution Buffer 1	8
Elution Buffer 2	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 series 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)

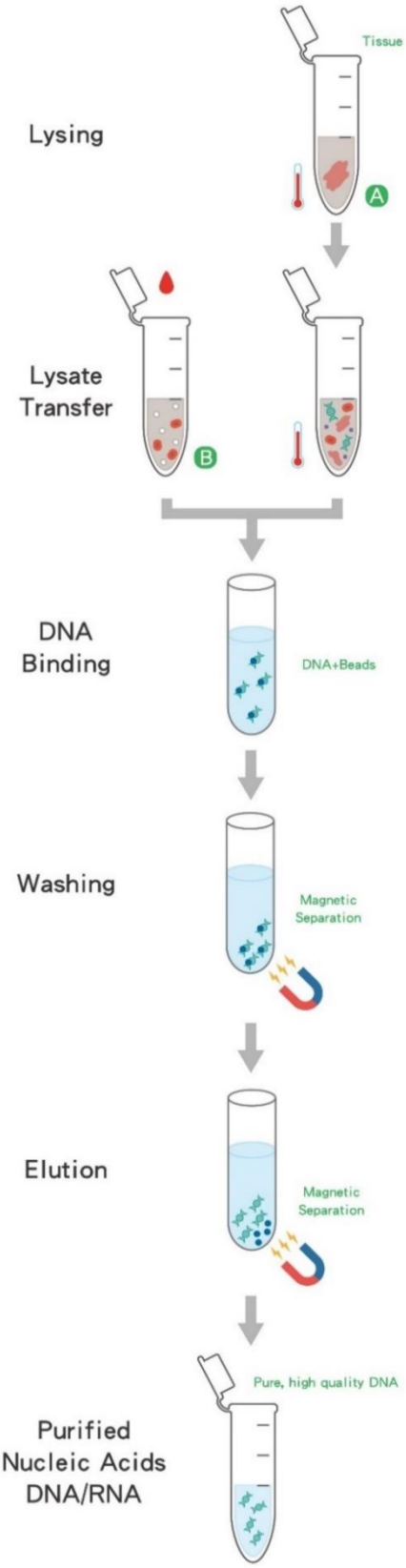
Warnings and Precautions

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.

B Perform certain pretreatment process before extraction.

Before Starting

Preparation of sample materials

The purification procedure is optimized for the use of appropriate value of human whole blood, dried blood, hair root, tissues, saliva and other forensic samples.

Whole Blood (fresh or frozen)	NOTE: To extract DNA from whole blood samples, please select and refer to ePure Blood DNA Extraction Kit 200 (E2001).
Clotted / Dried Blood	a. Cut the blood-contain range out, transfer pieces to sample tube. b. Add 400 µl BL2 buffer and 20 µl Proteinase K to sample. c. Incubate at 56°C for 15 minutes, vortex mixing several times during incubation or place sample in a thermomixer. d. Transfer all sample to filter column sitting in a sample tube. e. Short spin at 500 x *g*, 1 minute, to collect the clear flow-through in collection tube. f. Transfer 400 µl to the sample tube.
Forensic Surface and Contact Swabs	a. Allow the swab or brush to air-dry for at least 2 hours after collection. b. Carefully cut or break off the end part of the swab or brush into a 1.5 ml microcentrifuge tube, using an appropriate tool (e.g., scissors). c. Add 200-400 µl of BL2 buffer to the sample. d. Add 20 µl Proteinase K, vortex mixing for at least 10 seconds. e. *If processing brush samples, centrifuge the tube briefly (at 10,000 x *g* for 30 seconds) to force the brush to the bottom of the tube. f. Incubate at 56°C for 15 minutes, vortex mixing several times during incubation or place sample in a thermomixer. g. Pre-filter the digested lysate using a filter column to remove residual debris. h. Short spin at 500 x *g*, 1 minute, to collect the clear flow-through in collection tube. i. Transfer 200-400 µl to the sample tube.
Hair Root	Method 1 a. Place the hair sample in a 1.5 ml micro centrifuge tube b. Add 200 µl BL2 buffer to the sample. c. Add 20 µl Proteinase K and 10 µl 1M DTT solution*, and mix thoroughly by vortexing for at least 10 seconds. d. Incubate at 56°C for 15 minutes to 6 hours, vortex mixing several times during incubation or place sample in a thermomixer.

	e. (optional) Add extra 10 µl Proteinase K and 10 µl DTT and incubate at 56°C until the hair samples are completely dissolved. f. Spin the tube to remove drops from inside the lid. g. Pre-filter the digested lysate using a filter column to remove residual debris. h. Short spin at 500 x *g*, 1 minute, to collect the clear flow-through in collection tube. i. Transfer 200 µl to the sample tube. *Prepare 1 M DTT solution before processing the protocol (1 M is about 15% DTT (m/v)). Method 2 a. Place the hair sample in a 1.5 ml micro centrifuge tube. b. Add 200 µl BL2 buffer to the sample. c. Add 20 µl Proteinase K, mix thoroughly by vortexing for at least 10 seconds. d. Incubate at 56°C at least 15 minutes to overnight, vortex mixing several times during incubation or place sample in a thermomixer. e. Spin the tube to remove drops from inside the lid. f. Pre-filter the digested lysate using a filter column to remove residual debris. g. Short spin at 500 x *g*, 1 minute, to collect the clear flow-through in collection tube. h. Transfer 200 µl to the sample tube.
Human Tissues	a. Place tissue sample into a 1.5 ml micro centrifuge tube. b. Add 200-400 µl BL2 buffer and 20 µl Proteinase K to the sample, mix thoroughly by vortexing for 10 seconds. c. Incubate at 56°C for at least 2 hours, *vortex mixing several times during incubation or place sample in a thermomixer. d. *Incubation for longer time (e.g., overnight) is not making interferences of nucleic acid extraction. e. Spin the tube to remove drops from inside the lid. f. Pre-filter the digested lysate using a filter column to remove residual debris. g. Short spin at 500 x *g*, 1 minute, to collect the clear flow-through in collection tube. h. Transfer 200-400 µl to the sample tube.
Saliva	a. Place up to 50 µl saliva in a 1.5 ml micro centrifuge tube. b. Add 200 µl BL2 buffer to the sample. c. Add 20 µl Proteinase K, and mix thoroughly by vortexing for 10 seconds.

	d.	Incubate at 56°C for 15 minutes, vortex mixing several times during incubation or place sample in a thermomixer.
	e.	Spin the tube to remove drops from inside the lid.
	f.	Transfer 200 µl to the sample tube.
Sperm Stains	a.	Place the forensic sample in a 1.5 ml centrifuge tube.
	b.	Add 200-400 µl BL2 buffer to the sample.
	c.	Add 20 µl Proteinase K, and mix thoroughly by vortexing for 10 seconds.
	d.	Incubate at 56°C for 15 minutes, vortex mixing several times during incubation or place sample in a thermomixer.
	e.	Spin the tube briefly to remove drops from inside the lid.
	f.	Pre-filter the digested lysate using a filter column to remove residual debris and mucus.
	g.	Short spin at 500 x *g*, 1 minute, to collect the clear flow-through in collection tube.
	h.	Transfer 200-400 µl to the sample tube.
Chewing Gum	a.	Place the chewing gum sample in a 1.5 ml micro centrifuge tube.
	b.	Add 200 µl BL2 buffer to the sample.
	c.	Add 20 µl Proteinase K, and mix thoroughly by vortexing for 10 seconds.
	d.	Incubate at 56°C for 15 minutes, vortex mixing several times during incubation or place sample in a thermomixer.
	e.	Spin the tube briefly to remove drops from inside the lid.
	f.	Transfer 200 µl to the sample tube.
Cigarette Butts	a.	Place the cigarette butt sample in a 1.5 ml micro centrifuge tube.
	b.	Add 200 or 400 µl BL2 buffer to the sample.
	c.	(Check if the sample has absorbed buffer BL2, if necessarily add more Buffer BL2 to the sample).
	d.	Add 20 µl Proteinase K, and mix thoroughly by vortexing for 10 seconds.
	e.	Incubate at 56°C for 15 minutes, vortex mixing several times during incubation or place sample in a thermomixer.
	f.	Spin the tube briefly to remove drops from inside the lid.
	g.	Transfer 200 µl to the sample tube.
Stamps, Envelopes	a.	Add 200-400 µl BL2 buffer to the sample. (Check if the sample has absorbed BL2 buffer, if necessarily add more BL2 buffer to the sample).
	b.	Add 20 µl Proteinase K, and mix thoroughly by vortexing for 10 seconds.
	c.	Incubate at 56°C for 15 minutes, vortex mixing several times during incubation or place sample in a thermomixer.

d. Spin the tube briefly to remove drops from inside the lid.
e. Transfer 200-400 µl to the sample tube.

Note:

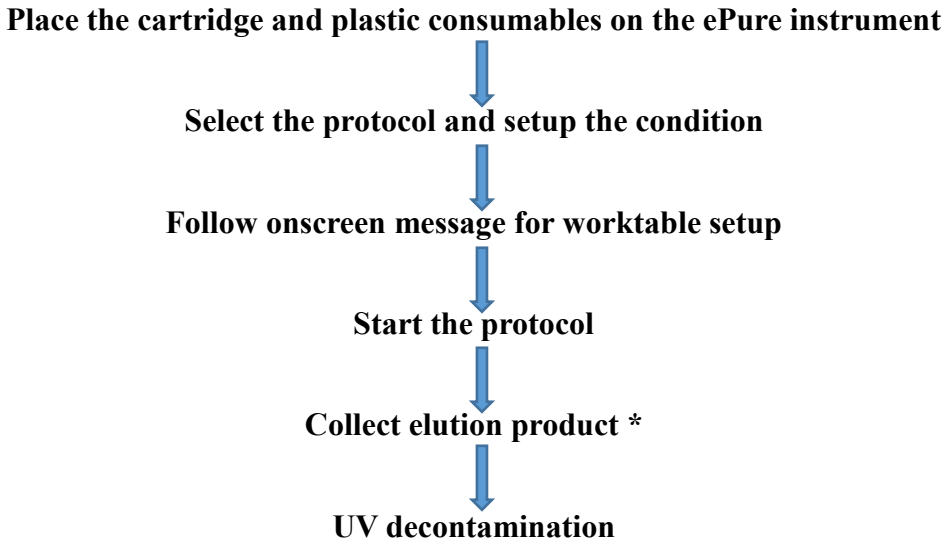
DNA quality will decrease with time or after multiple freezing - thawing repetitions. For longer storage time, samples should be frozen at -20°C or lower and avoid freezing-thawing repetitions. Thaw samples at room temperature (15-25°C) and process the sample immediately after equilibration to room temperature. **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.

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

Sample type	Starting material per sample	Elution Volume
Whole Blood (fresh or frozen)	100-400 µl	50-200 µl
Clotted / Dried Blood	400 / 3 card punches* * Using a single-hole paper punch to Cut 3 mm (1/8 inch) diameter of punches from a dried blood spot.	
Forensic Surface and Contact Swabs	200-400 µl	
Hair Root	100-400 µl / two or three 0.5-1 cm from the root ends of plucked hair samples	
Human Tissues	100-400 µl / up to 40 mg tissue	
Saliva	100-400 µl / 50 µl or more volume of saliva	
Sperm Stains	100-400 µl / 5-10 µl or 1 cm^2 of the forensic sample	
Chewing Gum	200-400 µl / up to 40 mg of chewing gum cut into small pieces	
Cigarette Butts	200-400 µl / approximately 1 cm^2 paper from the end of the cigarette or filter	
Stamps, Envelopes	200-400 µl / a 0.5-2.5 cm^2 piece of postage stamp or envelope	

Procedure of ePure System Procedure

Workflow of ePure operation



* Output the bench record (option)

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

Purification Protocol

1	Turn on the Instrument	a. b.	Turn ON the power switch - and wait for the screen to turn ON. Login and show the Home Page.
2	Load new Consumable(s) and Cartridge(s)	a. b. c. d. e. f.	Open the door and remove the sample rack from the instrument. Open the Tip-Holder Lid. 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). Close the Tip-Holder Lid. Paste the Barcode sticker on the Elution Tubes (optional). Place 6 Sample Tubes and 7 Elution Tubes into the Sample Rack.
3	Transfer samples into instrument	a. b.	Transfer appropriate volume of sample into sample tubes on sample rack. Put back the sample rack into the instrument and Close the door.
4	Program Set up	a. b.	Select the appropriate protocol program on the instrument. Press **NEXT**. 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 40-45 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 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

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	14 Feb. 2022	New document release