ePure Tissue DNA Extraction kit

Instructions for Use (Handbook)

REF E2004

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

Contents

Intended Use

The ePure Tissue DNA Extraction Kit provides a complete set of reagents and consumables for the automated purification of DNA (total nucleic acids) from solid Animal Tissue(s), dried swap and dried blood 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 Tissue DNA Extraction Kit
Catalogue Number	E2004
Product Overview	The ePure Tissue DNA Extraction Kit is designed to extract DNA from solid animal tissue(s), dried swap and dried blood. 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	2004 TISSUE DNA
Processing Time	ePure: 45 minutes

Kit Contents and Storage

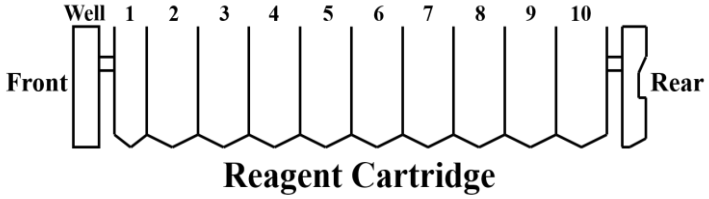
Shipping and Storage	The Kit is shipped at room temperature. Upon receipt, store the Kit at room temperature. All Kit components are stable when stored properly until the expiration date shown on the kit box.
Kit Content	The components supplied in the Kit are listed below. Sufficient reagents are supplied 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
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 1	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 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)

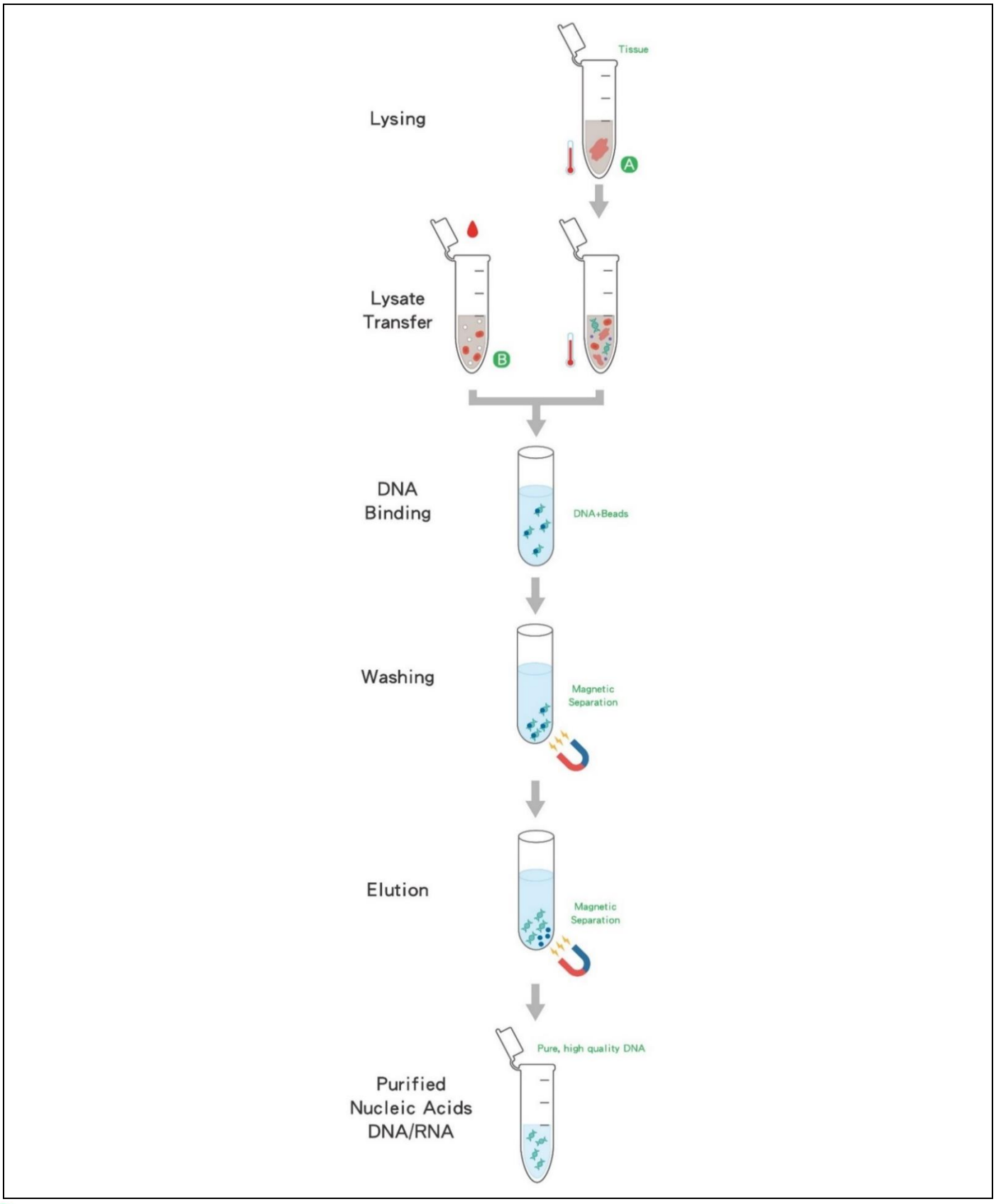
Warnings and Precautions

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 solid Animal Tissue(s), dried swap and dried blood samples as below table

Solid Animal Tissue(s)	a. Transfer the tissue to a 1.5 ml microcentrifuge tube. Cut tissue into small pieces or use a homogenizer to increase lysis efficiency and increase DNA yield. b. Add 220-440 µl of BL2 buffer to each sample and ensure that the tissue pieces are completely immersed in the buffer. c. Dispense 20 µl of proteinase K solution into each sample tube and vortex to mix. d. Incubate in a shaking water bath or thermomixer at 55°C until the tissue is completely dissolved. If you do not have a shaker/mixer device, vortex or mix the sample every 5 min, until the tissue pieces dissolve. The lysis time depends on the type of tissue to be treated. The lysis is usually completed within 1-2 hours. However, overnight lysis is possible and does not affect the preparation. * If the tissue cannot be completely dissolved, a larger amount than the recommended BL2 buffer / proteinase K mixture is required. e. Incubate the lysate at 70 °C for 10 minutes to heat inactivate the activity of proteinase K. f. **Optional**: Add DNase-free RNase A to degrade RNA present in the sample and minimize RNA contamination in the purified DNA sample. g. **Optional**: Before DNA extraction, pre-filter the digested tissue lysate using a filter column to remove residual debris and mucus. This will increase DNA production (20-100%). h. Spin down the treated lysate and transfer 200 µl into Sample Tube. *If the sample volume is lower than described, please complete with an appropriate volume of BL2 buffer.
Dried Swab Sample(s) e.g., Buccal cells.	a. Use a suitable tool (such as scissors) to carefully cut or break the end of a swab or brush into a 1.5 ml microcentrifuge tube. b. Add 220-440 µl of BL2 buffer to each sample and ensure that the sample pieces are completely immersed in the buffer. Dispense 20 µl of proteinase K solution into each sample tube and vortex to mix. *If using a buccal cell brush sample, centrifuge the tube briefly at 10,000 x *g* for 30 seconds to sink the brush into the bottom of the

	tube. c. Incubate in a shaking water bath or thermomixer at 55°C until the sample is completely dissolved. If you do not have a shaker/mixer device, vortex or mix the sample every 5 min, until the sample dissolve. The lysis time depends on the type of tissue to be treated. The lysis is usually completed within 1-2 hours. However, overnight lysis is possible and does not affect the preparation. d. Incubate the lysate at 70°C for 10 minutes to heat inactivate the activity of proteinase K. e. Spin down the lysate briefly to collect drops from the lid. f. Remove debris of swab or brush from the tube. Use clean forceps to squeeze the liquid from the residue of the swab or brush into the tube again to obtain the maximum sample volume. g. Transfer 200 µl supernatant into Sample Tube. *If the sample volume is lower than described, please complete with an appropriate volume of BL2 buffer.
Dried Blood Sample(s)	a. Collect 70 µl of each blood sample and gently apply to filter paper. Allow the specimen to fully air dry horizontally at room temperature. *Untreated blood or blood with anticoagulants (such as EDTA, ACD or heparin) also can be used. b. Collect four 3 mm diameter discs from the dried blood-stained filter paper and transfer them to a 1.5 ml microcentrifuge tube. c. Add 220-440 µl of BL2 buffer to each sample and ensure that the sample pieces are completely immersed in the buffer. d. Dispense 20 µl of proteinase K solution into each sample tube and vortex to mix. e. Incubate in a shaking water bath or thermomixer at 55°C until the sample is completely dissolved. If you do not have a shaker/mixer device, vortex or mix the sample every 5 min, until the sample dissolve. The lysis time depends on the type of tissue to be treated. The lysis is usually completed within 1-2 hours. However, overnight lysis is possible and does not affect the preparation. f. Incubate the lysate at 70°C for 10 minutes to heat inactivate the activity of proteinase K. g. Spin down the lysate briefly to collect drops from the lid. h. Transfer 200-400 µl supernatant into Sample Tube. *If the sample volume is lower than described, please complete with an appropriate volume of BL2 buffer.

Note:

In order to efficiently isolate genomic DNA from tissues, destruction and homogenization of sample material is essential. However, excessive disruption and homogenization will result in the shearing of high molecular weight genomic DNA.

Always prepare fresh tissue lysate and process immediately. When the DNA purification procedure is postponed, store the lysate at -20°C or lower and avoid freeze-thaw repetitions. Nucleic acid yield and quality will decrease with time or after multiple thawing.

To process RNA-rich tissues (e.g., high gene expression tissues, such as liver and tumors), add RNase after proteinase K incubation to digest RNA and increase DNA yield.

The final eluate contains total nucleic acid (DNA and RNA). RNA is not the major product in this kit (about 10%) and would degrade soon. If the RNA-free product is needed, please add RNase to treat the eluate. (For RNase treatment, follow the manufacturer instructions of the kit used in your lab.)

The requirements for sample preparation depend greatly on the type of raw material. Due to variations in consistency and viscosity, even similar sample types may require different processing methods. The following steps describe some suggestions for working with raw samples.

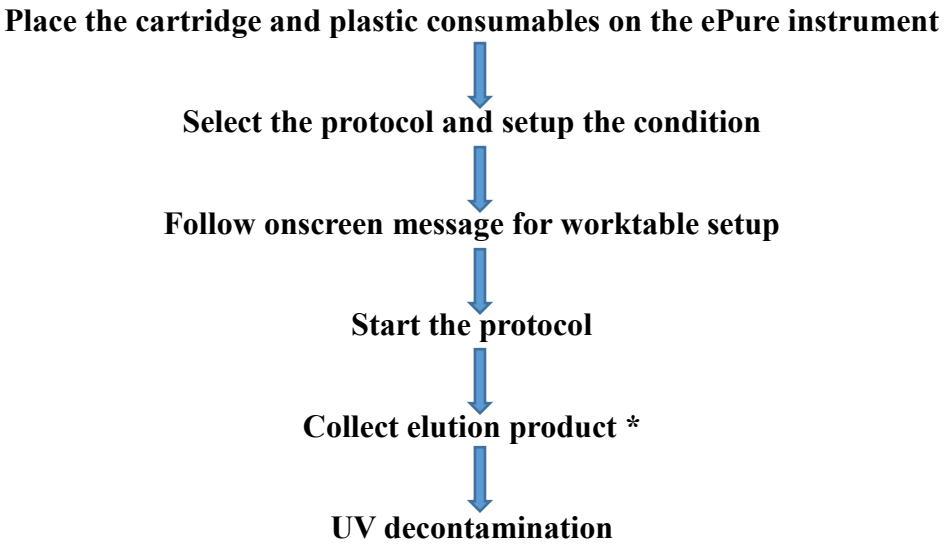
For **FFPE samples**, the ePure FFPE extraction kit (E2009) is recommended.

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

Sample type	Starting material per sample	Elution Volume
Solid Animal Tissue(s)	100-400 µl / 10-40 mg	50-200 µl
Dried Swab Sample(s) (e.g., Buccal cells)	100-400 µl / 1 swab or brush	
Dried Blood Sample(s)	100-400 µl / 4 discs*	

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.	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 program automatically until whole process is completed. c. At the end of the run (approximately 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