ePure Bacterial DNA Extraction Kit

Instructions for Use (Handbook)

REF E2006

Version: 1.0

Σ 48

For *in vitro* diagnostic use

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CE IVD

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 Bacterial DNA Extraction Kit provides a complete set of reagents and consumables for the automated purification of Bacterial DNA Extraction Kit provides reagents for fully automated and simultaneous purification of bacterial nucleic acids from human biological specimens, inactivation of pathogenic microorganism, bacterial pellet/colony from culture, clinical swab samples in liquid transport media, environment material (water, soil, etc.) with MagPurix 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 Bacterial DNA Extraction Kit
Catalogue Number	E2006
Product Overview	The ePure Bacterial DNA Extraction Kit is designed for the extraction of nucleic acids from human biological specimens, inactivation of pathogenic microorganism, bacterial pellet/colony from culture, clinical swab samples in liquid transport media, environment material (water, soil, etc.). The kit uses unique magnetic technology and in combination with ePure automatic instrument achieves superior product quality, consistent and high product yield and reproducible results. 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	2006 BACTERIAL DNA 2006 BACTERIAL RAPID
Processing Time	45-55 minutes RAPID: 28-36 minutes

Kit Contents and Storage

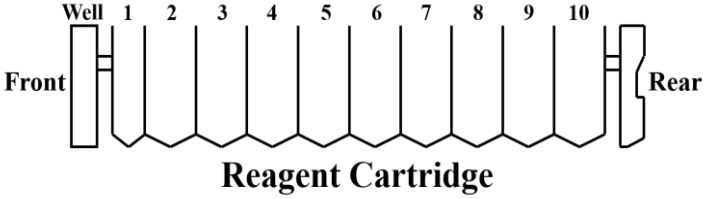
Shipping and Storage	The Kit is shipped at room temperature. Upon receipt, store the Kit at room temperature. All Kit components are stable when stored properly until the expiration date shown on the kit box.
Kit Content	The components supplied in the Kit are listed below. Sufficient reagents are supplied 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
BL2B 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.
Proteinase K Solution	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 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).

- 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

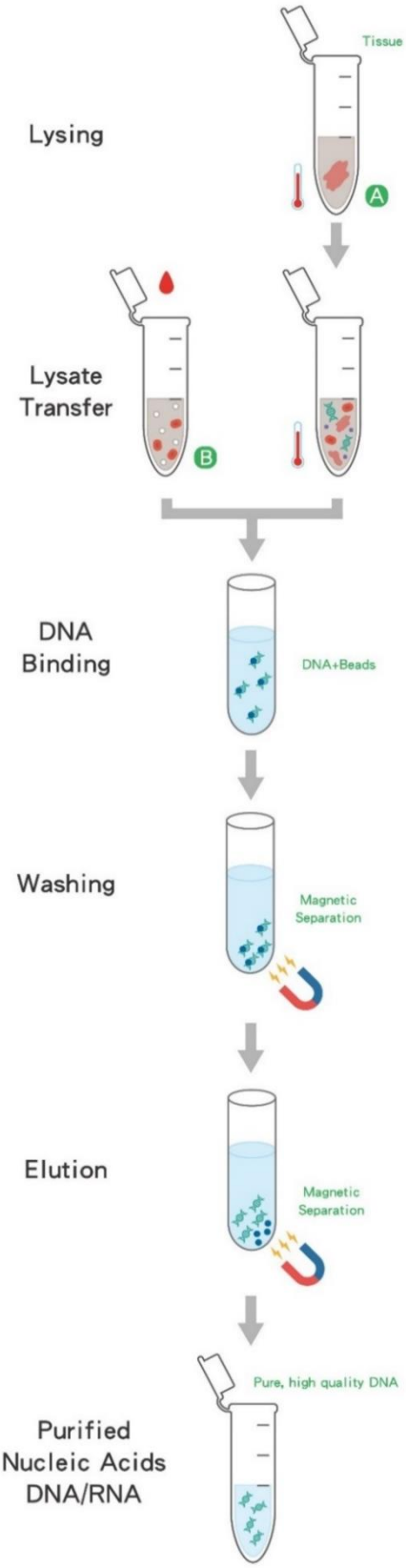
In accordance with the ISO certified EcoliDx quality management system, each kit is tested according to predetermined specifications to ensure consistent product quality.

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 of 200-400 µl serum, plasma*, CSF, pretreated urine or other cell-free body fluid samples.

Inactivation of pathogenic microorganism	Method 1 - liquid samples (e.g. liquid transport media) a. Collect samples in liquid transport media. b. Incubate for 10 minutes at 95°C. c. Centrifuge briefly to collect the complete sample volume at the bottom of the tube. d. Allow samples to cool down or chill on ice. e. Transfer 200-400 µl to sample tube. Method 2 - Non-liquid samples (e.g. dry swab) a. Place the samples in 1 ml PBS containing a common fungicide. b. Incubate for 30 minutes at room temperature. c. Incubate for 10 minutes at 95°C. d. Pellet microorganism by centrifugation at 14,000 x *g* for 10 minutes. e. Discard supernatant, and then re-suspend pellet in 220-440 µl BL2B Buffer. f. Take 200-400 µl suspension to sample tube.
Viscous samples	a. Collect viscous samples (e.g., BAL, sputum or other mucus specimen). b. Prepare a fresh DTT stock solution for liquefaction*. (e.g., 5× conc. DTT stock is about 0.75%) c. Adjust the final DTT concentration in the sample to 0.15 % by adding DTT stock solution. d. Incubate the sample (e.g., with shaking at 850 rpm for 30 minutes at 37°C) until it can be pipette easily. e. Pellet bacteria by centrifugation at 14,000 x *g* for 10 minutes. f. Discard supernatant, and then re-suspend the pellet in 220 µl BL2B Buffer. g. Transfer 200 µl to sample tube (Supplied in the kit). * The liquefaction could be done by using other solutions, such as NALC (N-Acetyl-L-Cysteine)-NaOH or other agents, which could digest mucous material.
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 BL2B 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 BL2B 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.	Spin down the treated lysate and transfer 200 µl into Sample Tube.
Cell-free body fluid(s)	a.	Pellet the bacteria by centrifugation at 14,000 x *g* for 15 minutes and discard the supernatant.
	b.	Resuspend the pellet in 220 µl BL2B Buffer.
	c.	Mix vigorously on a Vortex mixer for 5-10 seconds.
	d.	Transfer 200 µl supernatant into a Sample Tube.
Gram-positive bacterial species.	a.	Follow the regular homogenization* procedures in the laboratory.
	b.	For some sample types, DNA yield can be improved by performing this homogenization step prior to add BL2B Buffer and Proteinase K. * Especially for samples that contain particles (e.g., stool)
Bacterial colony	a.	Take 1-3 bacterial colony from culture plate with an inoculation loop and suspend in 220 µl of buffer BL2B by vigorous stirring.
	b.	Take 200 µl suspension to sample tube.
Bacterial suspension cultures	a.	Pipet 1 ml of bacterial culture into a 1.5 ml micro centrifuge tube and centrifuge at 5,000 x *g* for 5 minutes.
	b.	Discard supernatant
	c.	Add 220 µl Buffer BL2B to pellet and mix by vortexing for 5-10 seconds.
	d.	Take 200 µl suspension to sample tube.
Swab samples	Method 1 - Centrifuge	
	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.	Pellet bacteria by centrifugation at 14,000 x *g* for 10 minutes.
	d.	Discard supernatant, and then re-suspend pellet in 220 BL2B Buffer.
	e.	Take 200 µl suspension to sample tube.

	Method 2 - Centrifuge free f. Place the sample swab in 440 µl BL2B Buffer, incubate for 30 minutes at room temperature. g. Transfer 400 µl to sample tube.
Large volume liquid samples	a. Centrifuge* sample at 10,000-16,000 x *g* for 5-10 minutes to concentrate bacterial cells into a pellet. b. Discard supernatant and re-suspend the pellet in 220 µl Buffer BL2B Buffer.** c. Transfer 200 µl concentrated sample into sample tube. * Especially for samples that have low or unknown bacterial loads. (e.g., water collected from pool/river stream/tower, soil, urine.) ** If there were sand or other visible particle in the pellet, centrifuge again after BL2B Buffer treatment or filter out the dust is recommended.

Use the paraffin-embedded tissue sections as samples, we recommend using the by ePure FFPE DNA Extraction kit (E2009). If you use tissue as samples, we recommend using the ePure Tissue DNA Extraction kit (E2004).

Note:

The purification procedure is optimized for the use of 200-400 µl human biological specimens, inactivation of pathogenic microorganism, cultured bacterial pellet/colony suspend in liquid buffer, clinical swab samples in liquid transport media, environment material* (e.g., water, soil.).
*For large volume liquid samples with low or unknown bacterial content, e.g., water, soil, urine, or other, follow the recommended concentration procedure.

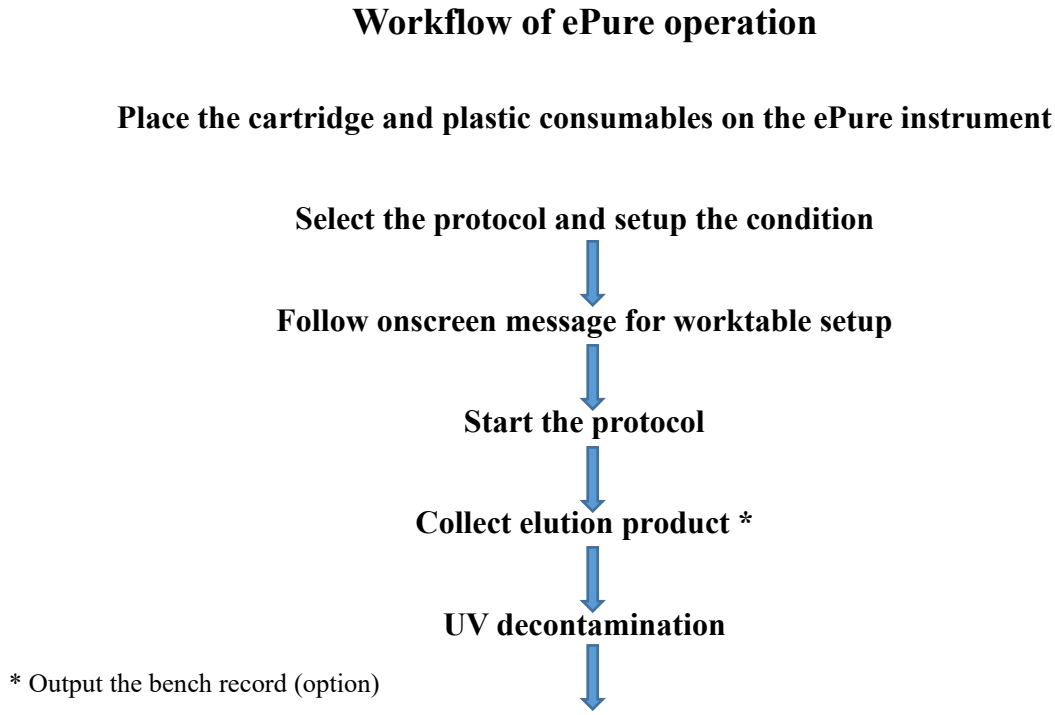
The buffer BL2B is specialized for bacterial cell wall lyse** (Supplied in the kit), use it to re-suspend the bacterial pellet or adjust sample volume before process extraction.
** For mycobacterium spp. (e.g., MTB), use buffer BL3 for bacterial cell wall lysis {BL3 buffer is supplied in the ePure TB DNA Extraction kit (E2008)}.

Use fresh sample (stored at 2-8°C for up to 6 hours) for extraction is recommended. Bacterial 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.

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)	200-400 µl / 1-30 mg	50-200 µl
Cell-free body fluids	200-400 µl	
Bacterial Pellet	200-400 µl NOTE: The use range is limited to up to 1×10^9 cells/ml (OD600 = 3.0) bacteria.	
Bacterial Colony	1-3 bacterial colony	
Bacterial Suspension Cultures	200-400 µl	
Swab Samples	200-400 µl	
Environment Material	200-400 µl *large volume liquid sample pretreatment	
Pretreated Urine	200-400 µl *large volume liquid sample pretreatment	

Isolation procedure using the ePure



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 1Reagent Cartridge, and all plastic disposables (2Reaction Chamber, 3Tip Holder, 4Piercing Pins, 5Filtered 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 6Sample Tubes and 7Elution 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 45-55 minutes) (**RAPID**: 28-36 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 DNA

Purified genomic DNA can be stored short-term at 2-8 ° C.
Long-term at -15 ° C to -30 ° C or -65 ° C to -90 ° C.

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 DNA quality or yield	Deterioration or contamination of reagents.	Please ensure that the reagents of kit are still in the effective use 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 times after arrival. If either Reagent or Buffer precipitates 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	The elution volume can be reduced

	used	proportionally.
	The eluent of final product(s) is not enough.	Please collect issue information and provide it to your Support Representative / Technical Support as soon as possible.
Clogged 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