ePure Plant DNA Extraction Kit

Instructions for Use (Handbook)

REF E2014

Version: 1.1

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

Contents

Intended Use

The ePure Plant DNA Extraction Kit provides a complete set of reagents and consumables for the automated purification of plant tissue and Yeast 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 Plant DNA Extraction Kit
Catalogue Number	E2014
Product Overview	The ePure Plant DNA Extraction Kit is designed to extract the genomic DNA from 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	2014 PLANT DNA
Processing Time	40-45 minutes

Kit Contents and Storage

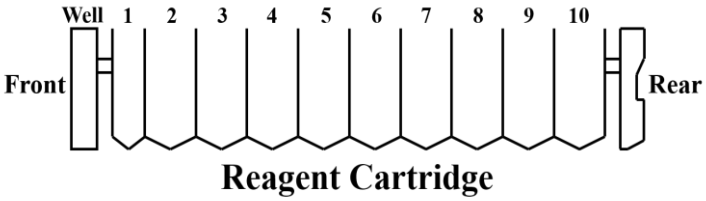
Shipping and Storage	The Kit is shipped at room temperature. Upon receipt, store the Kit at room temperature. All Kit components are stable when stored properly until the expiration date shown on the kit box.
Kit Content	The components supplied in the Kit are listed below. Sufficient reagents are supplied 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
Filter Column	50 pcs
Collection Tube	50 pcs
RNase A, 10 mg / mL (0.5 mL)	1 pc
PLA Buffer (25 mL)	1 pc
PLB 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 2	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).

- 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

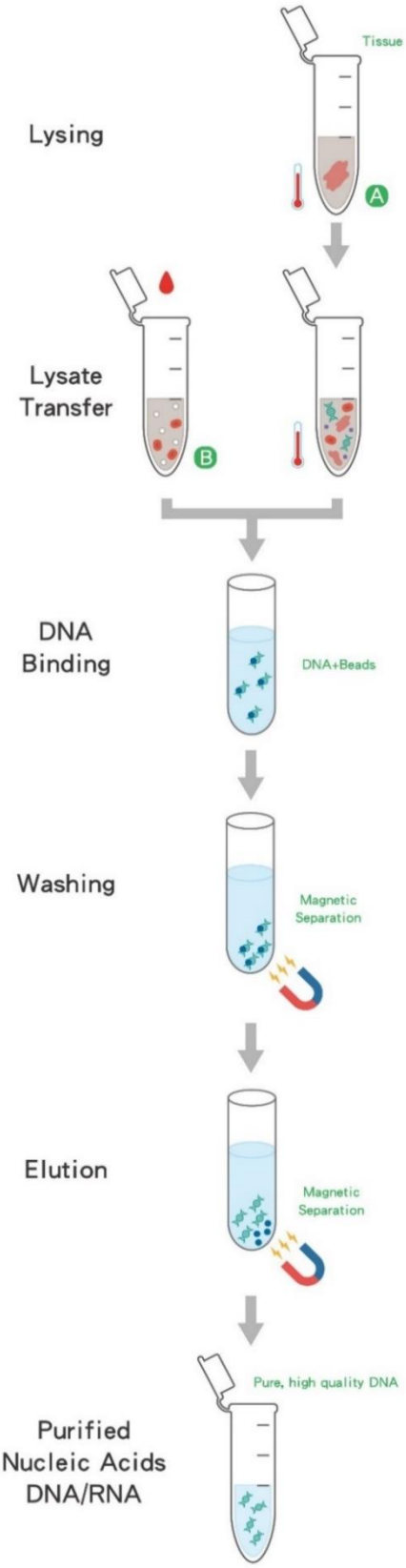
In accordance with the ISO certified EcoliDx quality management system, each kit is tested according to predetermined specifications to ensure consistent product quality.

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 appropriate samples as below table.

Plant tissue	a.	Perform homogenization by using proper homogenizer.
	b.	Add 440 µl lysis buffer to sample.
	c.	Vortex vigorously.
	d.	Incubate the mixture at 65°C, 10 minutes in a thermomixer (set at 1000 rpm) or vortex several times during incubation in the heat block or water bath.
	e.	Pre-filter the digested lysate using a filter column to remove residual debris.
	f.	Short spin at 6.000 x *g* to collect the clear flow-through in collection tube.
	g.	Add 10 µl RNase A, mix well, incubate for 10 minutes at room temperature.
	h.	Transfer 400 µl to sample tube.
Yeast / Suspension culture	a.	Centrifuge at 6.000 x *g*, 3 minutes.
	b.	Remove supernatant.
	c.	Add 440 µl lysis buffer, vortex mixing 30 seconds.
	d.	Incubate the mixture at 65°C, 10 minutes in a thermomixer (set at 1000 rpm or vortex several times during incubation in the heat block or water bath.
	e.	Pre-filter the digested lysate using a filter column to remove residual debris.
	f.	Short spin at 6.000 x *g* to collect the clear flow-through in collection tube.
	g.	Transfer 400 µl to the sample tube.
Yeast / Colony	a.	Take 1-3 colony from culture plate with an inoculation loop and suspend in 440 µl of lysis buffer by vigorous stirring.
	b.	Incubate the mixture at 65°C, 10 minutes in a thermomixer (set at 1000 rpm) or vortex several times during incubation in the heat block or water bath.
	c.	Pre-filter the digested lysate using a filter column to remove residual debris.
	d.	Short spin at 6.000 x *g* to collect the clear flow-through in collection tube.
	e.	Take 400 µl suspension to sample tube.

Note:

After harvesting plant tissues, it should be frozen in liquid nitrogen if not be used immediately. It

can then be stored at -80°C. Alternatively, tissue can be dried or lyophilized after harvesting to allow storage at room temperature (15-25°C). To ensure DNA quality, samples should be completely dried within 24 hours of collection. If possible, it is preferable to collect young materials (e.g., leaves, needles) since they contain more cells per weight and therefore result in higher yields.

When working with fungi, harvest mycelium directly from a culture dish or from liquid culture. For liquid culture, first pellet cells by centrifugation. Remove the supernatant completely before disruption and lysis. Fresh, frozen, or freeze-dried fungal material can be used.

The disruption method may require optimization to ensure maximum DNA yield and quality. Complete and quick disruption of starting material is essential to ensure high DNA yields and to avoid DNA degradation.

Before DNA extraction, plant material shall be first mechanically disrupted with lysis buffer*. After Homogenization, remove the debris and other precipitations by spin through the filter membrane. Collect the clear flow-through and incubate with RNase A to digest the RNA in the sample before DNA extraction.

* We provide two lysis buffers: **PLA Buffer** and **PLB Buffer** for dealing with different tissue types. Before extraction a new tissue type, try these lysis buffer for getting the optimized lysis procedure and a better DNA yield. If the precipitation formed in the lysis buffer, warm it at 65 °C before use.

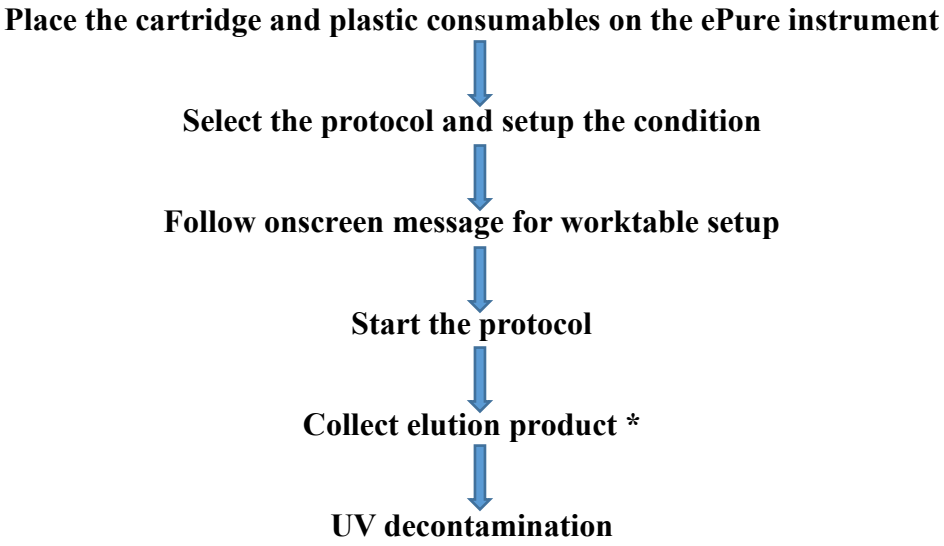
The suggested starting material and elution volume ranged for each nucleic acid extraction.

Sample type	Starting material per sample	Concentration (ng/μl)	Elution Volume
Plant tissue			
Soybean	100-400 μl / 100 mg seed	5-12 (PLA) / 50-80 (PLB)	50-200 μl
Rice	100-400 μl / 20 mg seed	5-8 (PLA) / 15-25 (PLB)	
Arabidopsis	100-400 μl / 100 mg leaf	2-5 (PLA) / 5-7 (PLB)	
Tomato		20-40 (PLA)	
Corn		10-15 (PLA) / 25-60 (PLB)	
Tectaria		5-10 (PLA)	
Aspidistra		3-6 (PLA)	
Pharius		20-25 (PLA) / 50-100 (PLB)	
Zingiber -		3-8 (PLA) / 20 -25 (PLB)	
Yeast			
Suspension Cultures	100-400 μl		50-200 μl
Colony	1-3 colony		

Isolation procedure using the ePure

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

Workflow of ePure operation



* Output the bench record (option)

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.
		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 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 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 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:	Please collect issue information (videos

	1. Inaccurate correction value 2. Spare parts or components damaged	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