MagPurix® Total RNA Extraction Kit (ZP02015)

Instructions for Use (Handbook)

Version: 2.0

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.zinexts.com

Contents

Intended Use

The MagPurix® Total RNA Extraction Kit provides a complete set of reagents and consumables for the automated purification of total RNA from mammalian whole blood, human and animal tissues, cultured cells, plant tissue and yeast, using the 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	MagPurix® Total RNA Extraction Kit
Catalogue Number	ZP02015
Product Overview	The MagPurix® Total RNA Extraction Kit is designed to extract total RNA from mammalian whole blood, human and animal tissues, cultured cells, plant tissue and yeast using MagPurix® series automated instruments. The kit is applied with unique magnetic ZiBeads® technology, which achieves consistent and high product yield and reproducible results. The final product is suitable for a wide range of molecular biology applications, such as sequencing, genotyping, qPCR, ddPCR and NGS assays.
Applicable Instrument Model	All MagPurix® Instruments
Display Protocol Name on the Instrument	2015 TOTAL RNA
Applicable Instrument Firmware	Please check and download the latest firmware from www.zinexts.com
Processing Time	MagPurix® 12 series 45-55 minutes MagPurix® 24 series 45-60 minutes MagPurix® EVO series 35-40 minutes

Kit Contents and Storage

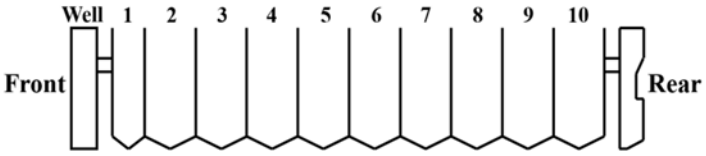
Shipping and Storage	The kit is shipped at room temperature. Upon receipt, store the kit at room temperature. All kit components are stable when stored properly until the expiration date shown on the kit box.	
Kit Content	The components supplied in the kit are listed below. Sufficient reagents are supplied 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
Proteinase K, 10 mg/ml (1 ml)	1 pc
Barcode Sticker (EVO only)	50 pcs

Reagent Cartridge Contents

Each Reagent Cartridge has 10 positions with 10 sealed well. Positions 1-10 contain wells that are filled reagents for this extraction 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

Equipment and Reagents to be Supplied by User

The following general laboratory equipment and consumables are required to perform the extraction. All laboratory equipment should be installed, calibrated, operated, and maintained according to the manufacturer's recommendations. The following table lists the required equipment and consumables.

For all purification procedures:
1. MagPurix® / MagPurix® EVO series instrument
2. 1.5 or 2.0 ml microcentrifuge tubes
3. Pipettes and filter tips
4. Phosphate-buffered saline (PBS, may be required for diluting samples)
Optional:
1. Plastic consumables, DNase-free RNase A (to minimize RNA content)
2. RNAlater
3. RNase Zap

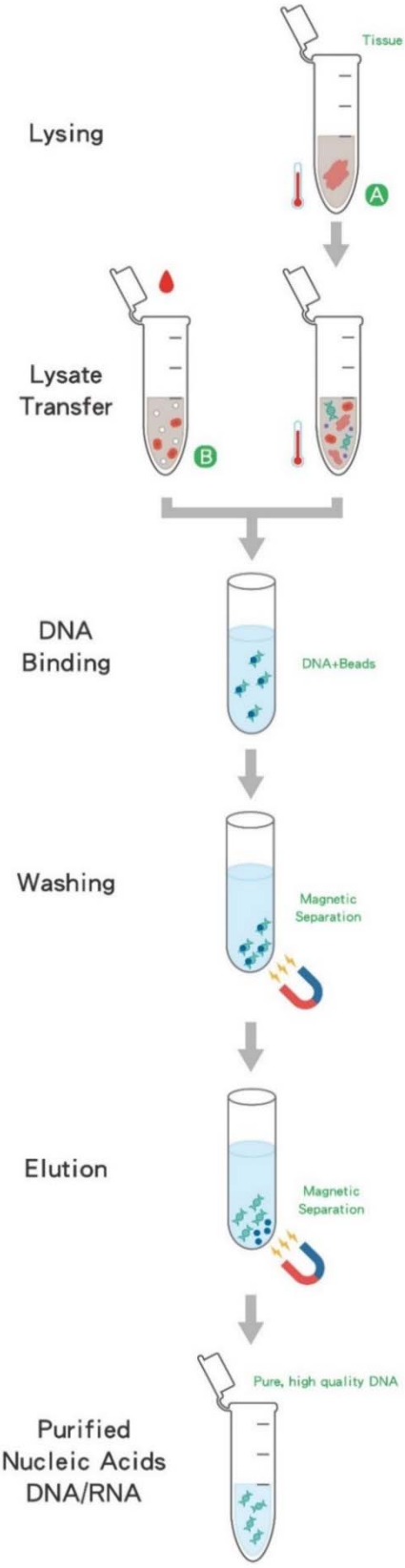
Warnings and Precautions

For research 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 nucleic acid 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 (SDSs). These are available online at MSDS (Material Safety Data Sheets) - Downloads | Zinexts Life Science Corp.

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

Purification Principle



Ⓐ Perform certain pretreatment process before extraction.
Ⓑ Transfer sample to extraction directly.

Things to Do Before Starting

Pretreatment of Samples

The purification procedure is optimized for the use of appropriate samples as below table

Mammalian Whole Blood	a. Prepare fresh 1X RBC lysis buffer. b. Add ice-cold RBC lysis buffer to blood sample in 2:1 ratio. c. Invert 3-5 times, incubate on ice for 10-15 minutes. d. Centrifuge at 500 x *g*, 10 minutes, 4°C. e. Remove supernatant. f. Resuspend the pellet with 220 µl 4°C RLA Buffer. g. Add 20 µl proteinase K h. Transfer 200µl into each Sample Tube.
Human and Animal Tissue	a. Add 220-440 µl 4°C RLA lysis buffer to tissue; make sure the sample is completely immersed in the buffer. * If the tissue cannot be completely immersed, larger amount (up to 440 µl) of 4°C RLA Buffer is required. b. Lysed the tissue by homogenizer. c. Spin the lysate to collect the liquid on the bottom 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 at 4°C. f. Add 20 µl proteinase K g. Transfer 200-400 µl into each Sample Tube.
Suspension Culture	a. Harvest cell culture. b. Centrifuge at 300x *g* for 5 minutes at 4°C. c. Remove supernatant completely. d. Resuspend cell pellet with 220 µl 4°C RLA Buffer. e. Vortex for 10 seconds. f. Add 20 µl proteinase K g. Transfer 200 µl into each Sample Tube.
Monolayer Culture	Method 1 a. Trypsinize the cells. b. Harvest the cell in PBS. c. Centrifuge at 300 x *g* for 5 minutes at 4°C. d. Remove the supernatant. e. Resuspend the pellet with 220 µl 4°C RLA Buffer. f. Vortex for 10 seconds. g. Add 20 µl proteinase K h. Transfer 200 µl into each Sample Tube. Method 2 i. Scrape the cells with 220-440 µl 4°C RLA Buffer. j. Vortex for 10 seconds. k. Transfer 200-400 µl into each Sample Tube.
Plant tissue/ Yeast	a. Add 220-440 µl 4°C RLA or RLB Buffer to the sample; make sure the sample is completely immersed in the buffer. b. Homogenize the 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 at 4°C.
	e.	Add 20 µl proteinase K
	f.	Transfer 200-400 µl into each Sample Tube.
DNA-free RNA extraction	a.	Perform total RNA program extraction.
	b.	Add 2 µl DNase in the eluate.
	c.	Incubate at 37°C for 10 minutes.
	d.	Transfer the mixture to a new Sample Tube.
	e.	Start the "Total RNA" extraction protocol again.

Note:

- If performing DNA-free protocol, prepare DNase before extraction. Place 10µl DNase in the first elute product.
- Wear clean gloves and keep work area, pipettes and reagents free of virus, bacteria and nuclease contamination to use RNase-free filter tip. Using RNase Zap® to clean the surface of bench, equipment and pipettes is one of the easiest ways to remove the RNase contaminations in the work area.
- Using RNA stabilized reagent (e.g., RNA-*later*®) to treat sample is one of the best ways to protect the RNA if the sample cannot be processed in a RNase-free working area.
- Two RL Lysis Buffers (RLA and RLB) are supplied in the kit for treating different tissue types. User could try both lysis buffers to get the optimized extraction results.

Reagent	Description	Preparation
β-Mercaptoethanol (β-ME)	β-ME reduce disulfide bonds and irreversibly denature the RNase, eliminating RNase released during cell lysis.	Add 10µl 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 to 1/10 freshly 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

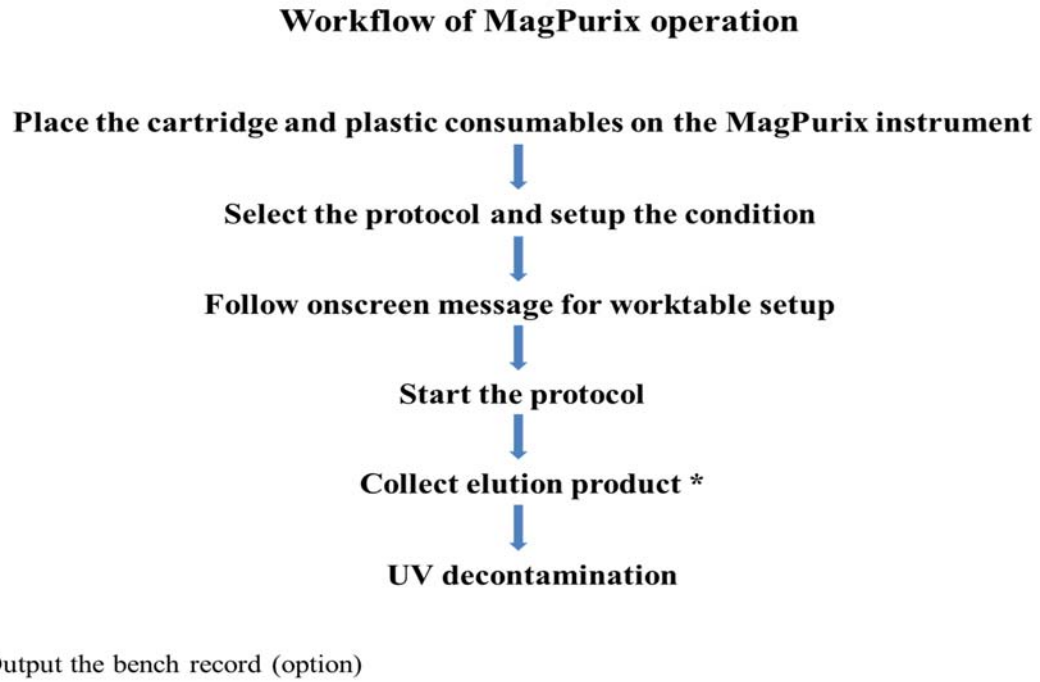
See the below table for the suggested starting material and elution volume range for each nucleic acid extraction

Sample type	Starting material per sample	Elution Volume
Mammalian Whole Blood	200 – 400 µl (total WBC number is about 1 x 10^6 cells) NOTE: 1. Use the fresh whole blood sample for isolation (within 4 hours, on ice). Freezing blood is not	50-300 µl (EVO 50-200 µl)

	allowed. The blood sample should be collected in the presence of an anticoagulant, preferably EDTA, although other anticoagulants such as citrate, heparin, or ACD (acid citrate dextrose) can also be used. 2. For optimal results, blood samples should be processed within a few hours of collection and keep at 4℃. 3. Perform Erythrocyte (RBC) lysis procedure before extraction. 4. If the whole blood samples with extremely high WBC numbers (more than 10000 cells) or concentrated PBMCs (Peripheral Blood Mononucleated Cells) are used, the input volume for extraction is recommended to be decreased (total WBC number should be less than 10^6 cells).	
Animal Tissue	200-400 µl /10-40 mg NOTE: 1. To prevent degradation by intracellular RNase, it is important that tissues are either flash-frozen in liquid nitrogen or stored at -70°C, or processed immediately following excision. 2. Using RNA stabilized reagent (e.g., RNA-later or RLA buffer) to treat tissue is another option to protect the RNA if the sample cannot be frozen immediately. Frozen tissue should not be thawed during handling (e.g., weighing). Keeping sample on ice during cutting or homogenization with RLA Buffer is recommended. 3. After homogenization, use filter column (supplied in the kit) to remove the insoluble and viscous materials of the lysates.	
Cultured Cell	200-400 µl /up to 5 x 10^6 cells NOTE: 1. Cells or isolated blood cells can be collected as pellets and either flash-frozen in liquid nitrogen and stored at -70°C, or processed immediately. Add RLA Buffer to resuspend pellet for extraction 2. Alternatively, samples can be stored at -70°C in RLA Buffer after disruption and homogenization. Samples frozen in this way are stable for months.	
Plant tissue Yeast	200-400 µl /up to 100 mg NOTE: 1. Up to 100 mg of sample should be placed in liquid nitrogen or frozen, then add lysis buffer (RLA or RLB Buffer) to homogenizer.	

	2. Most plant cells use RLA Buffer for disruption and denaturing sample 3. However, some tissues, such as milky endosperm of maize or mycelia of filamentous fungi, solidify in RLA Buffer, making the extraction of RNA impossible. In these cases, RLB Buffer should be used instead. 4. After adding lysis buffer (RLA or RLB), samples are placed into homogenizer for homogenization 5. After homogenization, use filter column (supplied in the kit) to remove the insoluble and viscous materials of the lysates.	

Procedure of MagPurix System



* Output the bench record (option)

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

Purification Protocol - MagPurix® series

1	Turn on the Instrument	a. Turn on the power switch and wait for the screen to turn on.
2	Load new Consumable(s) and Cartridge(s)	a. Open the door and remove the Sample Rack from the instrument. b. Load 1 Reagent Cartridge, and all plastic disposables (2 Reaction Chamber, 3 Tip Holder, 4 Piercing Pins, 5 Filter Tips and other components presented in the kit intended to use). Place 6 Sample Tubes and 7 Elution Tubes into the Sample Rack.
3	Load the Samples	a. Transfer appropriate volume of sample and add 20 µl proteinase K into each Sample Tube on the Sample Rack.

		b. Put the Sample Rack back into the instrument and close the door.
4	Program Set up	a. Scan the protocol barcodes to select the purification protocol, sample volume and elution volume.
5	Start Extraction	a. Follow the instructions displayed on the screen to double-check the operating steps being completed before program running. b. Press "**ENTER**" to start the experiment. Instrument will run the protocol program automatically until the whole process is completed. c. At the end of the run (approximately 12 series 45-55 minutes, 24 series 45-60 minutes), instrument alarms briefly.
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 immediately at -70°C before performing downstream analysis. d. Discard the used cartridges and all plastic consumables into biohazard waste. *Do not reuse the cartridges. e. If you are not using the instrument immediately, place the Sample Rack back to the workplace, close the instrument door and press "Start" button for 2 seconds to enter sleep mode. Moreover, if the instrument will not be used in a long time, please turn off the power switch.

Purification Protocol - MagPurix® EVO series

1	Turn on the Instrument	a. Turn on the power switch and wait for the screen to turn on. a. Login the instrument and enter 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 presented in the kit intended to use). d. Close the Tip-Holder Lid. e. Paste the barcode stickers on Elution Tubes. a. Place 6 Sample Tubes and 7 Elution Tubes into the Sample Rack.
3	Load the Samples	a. Transfer appropriate volume of sample into each Sample Tube on the Sample Rack. a. Put the Sample Rack back into the instrument and close the door.
4	Program Set up	a. Select the appropriate protocol program on the instrument. Press **NEXT**. b. Select the appropriate Sample Volume and 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 is expired, the next step cannot be performed.

		a. Follow the instructions on the screen to double-check the operating steps being completed before running the program. Press **NEXT**.
5	Start Extraction	a. Check "**PROGRAM CONFIRMATION**" on the screen. b. Press "**START**" to start the experiment. Instrument will run the protocol program automatically until the 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 want to perform the same protocol, press "**RERUN**" to perform the same experiment. If you do not need to 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 and all plastic consumables into biohazard waste. *Do not reuse the cartridges. e. If you are not using the instrument immediately, please put 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 in a long time, please turn off the power switch.

Troubleshooting

***This table is helpful for solving common problems. If you need other technical support, please contact Zinexts team (http://www.zinexts.com/index.php?lang=en) or 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 within the effective shelf-life 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 to 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 the 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 detailed information of the issue and provide it to your Support Representative/Technical Support as soon as possible.
	Kit stored under non-optimal conditions	Store kit at 15 to 25°C at all times upon arrival
	RNA degradation before extraction	1. Before extraction, protecting the sample from RNase digestion is very important. Use RNA-later or RLA buffer to protect sample is recommended. 2. Always use fresh sample and keep it in low temperature before extraction. If possible, immerse sample in RNA-later or PLA buffer in 4°C for 1 to 2 hours. Store sample in -70°C if not isolating RNA immediately.
	Reagents and samples not completely mixed	Always mix the sample tube well after addition of each reagent
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.	Use the appropriate controls for analysis. Check the positive control, negative control, water (NTC) and internal control to clarify the possible causes.
Low RNA yield	High levels of RNase activity	Be careful to create an RNase-free working environment
		Process starting material immediately or store it at -80°C until it can be processed.
		Use eluted RNA directly in downstream procedures or store it immediately at -80°C.
Instrument malfunction/abnormal sound	Abnormal consumables: 1. Deformed Filter Tips 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 detailed information of issue (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.
MagPurix® Blood DNA Extraction Kit 200	ZP02001
MagPurix® Blood DNA Extraction Kit 1200	ZP02002
MagPurix® Viral NA Extraction Kit	ZP02003
MagPurix® Tissue DNA Extraction Kit	ZP02004
MagPurix® Cultured Cell DNA Extraction Kit	ZP02005
MagPurix® Bacterial DNA Extraction Kit	ZP02006
MagPurix® HPV DNA Extraction Kit	ZP02007
MagPurix® TB DNA Extraction Kit	ZP02008
MagPurix® FFPE DNA Extraction Kit	ZP02009
MagPurix® Forensic DNA Extraction Kit	ZP02010
MagPurix® Viral Pathogen DNA Extraction Kit A	ZP02011
MagPurix® Viral Pathogen DNA Extraction Kit B	ZP02012
MagPurix® Viral RNA Extraction Kit	ZP02013
MagPurix® Plant DNA Extraction Kit	ZP02014
MagPurix® Total RNA Extraction Kit	ZP02015
MagPurix® Viral NA Extraction Kit LV	ZP02016
MagPurix® CFC DNA Extraction Kit	ZP02017
MagPurix® cfDNA Extraction Kit Plus	ZP02024
MagPurix® cfDNA Extraction Kit LV	ZP02025
MagPurix® Coronavirus RNA Extraction Kit	ZP02027

References

- Tan SC *et al.* J Biomed Biotechnol. (2009)

Symbols

The following symbols are used on labels and in Instructions for Use (IFU), in compliance with EN ISO 15223-1 standard.

Symbol	Explanation
CE	CE mark
IVD	For In Vitro Diagnostic Use
REF	Catalogue number

	Lot/Batch number
	Sufficient for [n] samples
	Instructions for Use
	Expiry date
	Storage temperature (15°C - 25°C)
	Manufacturer
	European Authorized Representative
	Caution

Limited Product Warranty

Zinexts Life Science 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.

Zinexts Life Science guarantees the performance of all products according to the specifications stated in our product literature. The purchasers/users must determine the suitability of the product for their particular use. We reserve the right to change, alter, or modify any product to enhance its performance and design.

This warranty limits Zinexts Life Science Corporation’s liability only to the cost of the product. 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
1.9	11. Aug. 2022	List of IVD symbols added. Suitable sample types updated.
2.0	19. Sep. 2022	1. Add Proteinase K product content 2. Add instructions for start material preparation 3. Troubleshooting: add new item