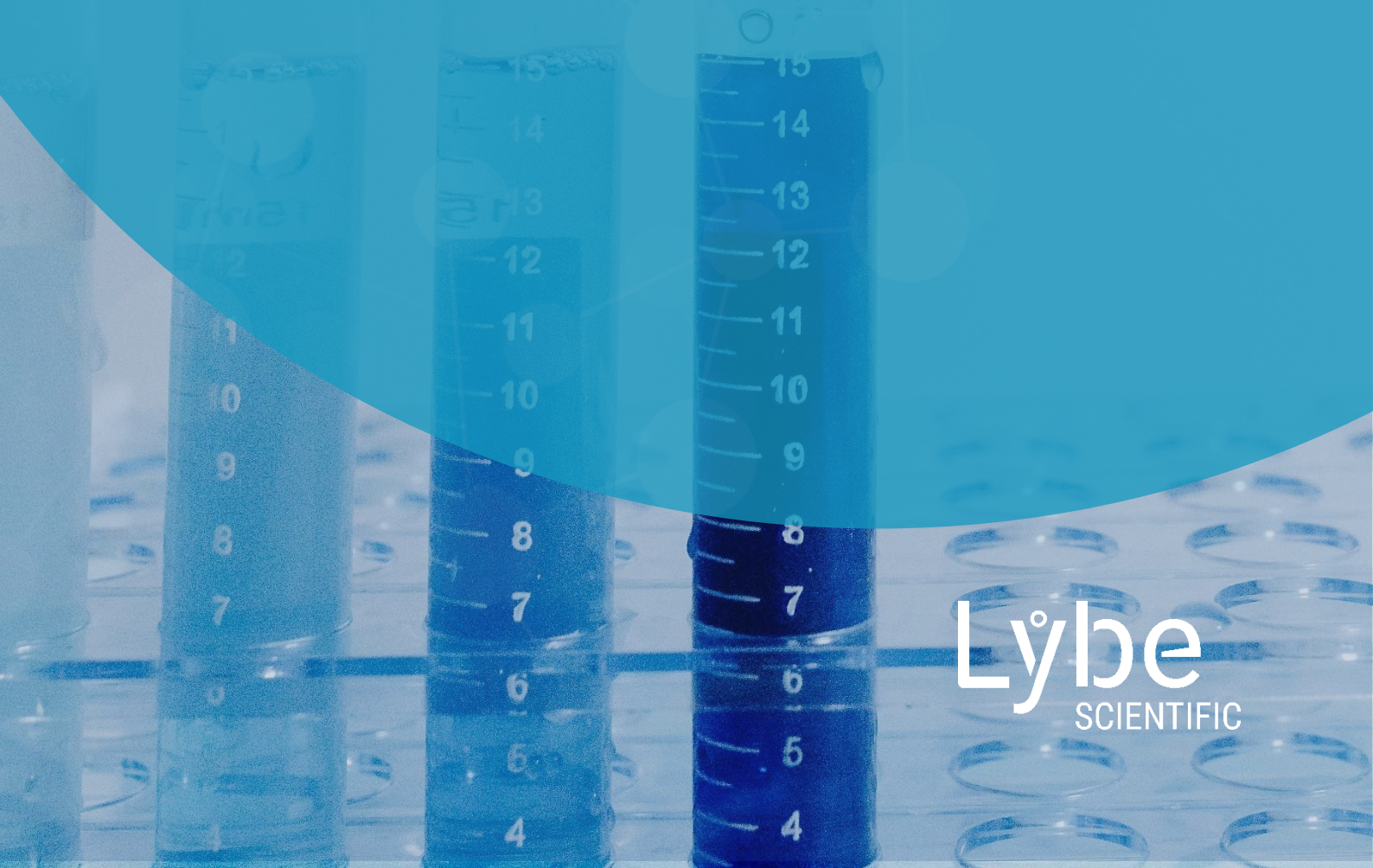
INSTRUCTIONS FOR USE

NAxtra™ Blood total nucleic acid extraction kit



Lybe SCIENTIFIC

NAxtra™ Blood total nucleic acid extraction kit

INSTRUCTIONS FOR USE

Catalog number: LSBL0048, LSBL0096

Revision 01

The customer is responsible for compliance with regulatory requirements that pertain to their procedures and uses of the product.

The information in this guide is subject to change without notice.

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Revision	Date	Description
01	21 February 2023	New document.

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Contents

General information

Intended use

NAxtra™ Blood total nucleic acid extraction kit is a magnetic bead-based technology intended for nucleic acid extraction from whole blood samples. The product should be used for isolation and purification of high-quality DNA and RNA from whole blood samples. The kit is intended for research use only.

Product information

NAxtra™ Blood total nucleic acid extraction kit (Cat. No. LSBL0048, LSBL096) specifically designed to recover nucleic acids from whole blood samples. The kit is based on magnetic bead technology, ensuring sensitive and high-quality recovery of nucleic acids for down-stream applications like for example qPCR, qRT-PCR and next-generation sequencing.

Specimen Collection and handling

This protocol has been optimized using EDTA as blood stabilizer during sampling.

Kit specifications

NAxtra™ Blood Total Nucleic Acid Extraction kit can be used by manual handling protocols as well as on common liquid handling instruments or automated magnetic separators. Typically, 96 samples can be purified in less than 25 minutes using the NAxtra™ Blood total nucleic acid extraction kit on a KingFisher™ Flex system. The actual procedure time depends on the configuration of the instrument and the magnetic separation system used.

The product is intended for research use only and include the following features:

- Protocol options for both manual and automated extractions
- Fast extraction of nucleic acid using automated liquid handing robots
- No need for carrier
- **Input volume: 100 µl whole blood is recommended**, but any input up to 200 µl can be extracted without adjusting volumes. Although 100-200 µl in-put could possibly result in gel-matrix formation (sample dependent) at elution step. Increasing elution volume could help prevent this.
- Elution volume of 50-200 µl

Shipping conditions

Products are shipped at ambient temperature.

Contents and storage

The NAxtra™ Blood total nucleic acid extraction kit contains sufficient reagents for 48 reactions (LSBL0048) and 96 reactions (LSBL0096) with 10 - 200 µl sample input volume. Review your assay documentation to determine optimal sample input volume. Review product labels for information regarding expiry dates.

Component Content	48 reactions (LSBL0048)	96 reactions (LSBL0096)	Storage
NAxtra™ LYSIS BUFFER	17 ml	34 ml	2-8°C
NAxtra™ MAGNETIC BEADS	1 ml	2 ml	
NAxtra™ Binding Buffer	4.8 ml	9.6 ml	
NAxtra™ Wash buffer 1/2	30 ml	60 ml	
NAxtra™ Proteinase K	0.5 ml	1 ml	
NAxtra™ Elution Buffer	10 ml	20 ml	

Required materials not supplied: Manual extraction

Item	Description
100% Isopropanol	Bead/Binding mix
100% Ethanol	Wash solution
Plastic consumables (tubes, plates, tips)	As appropriate
Pipettes	As appropriate
Vortex	As appropriate
Magnetic rack	Separation of beads from solution

Materials not supplied: Suggested materials for automated extraction using KingFisher™ Duo Prime Purification System

Item	Description
100% Isopropanol	Bead mix and wash solution
100% Ethanol	Wash solution
KingFisher™ Duo Prime Purification System with 12 Deep-well Head	ThermoFisher Scientific, Catalog number: 5400110
KingFisher™ 96 deep-well plate, v-bottom, polypropylene (for Duo Prime, Flex and Presto)	ThermoFisher Scientific, Catalog number: 95040450
KingFisher™ Duo 12 Deep-Well Tip Combs	ThermoFisher Scientific, Catalog number: 97003500
Nunc™ 96-Well Polypropylene Storage Microplates	ThermoFisher Scientific, Catalog number: 249946

In addition: General single- and multichannel pipettes and tips for 100-1000 µl as appropriate.

Materials not supplied: Suggested materials for automated extraction using KingFisher™ Flex Purification System

Item	Description
100% Isopropanol	Bead mix and wash solution
100% Ethanol	Wash solution
KingFisher™ Flex Purification System with 96 Deep-well Head	ThermoFisher Scientific, Catalog number: 5400630
KingFisher™ 96 deep-well plate, v-bottom, polypropylene (for Duo Prime, Flex and Presto)	ThermoFisher Scientific, Catalog number: 95040450
KingFisher™ Flex 96 Deep-Well Tip Combs	ThermoFisher Scientific, Catalog number: 97002534
Nunc™ 96-Well Polypropylene Storage Microplates	ThermoFisher Scientific, Catalog number: 249946

In addition: General single- and multichannel pipettes and tips for 100-1000 µl as appropriate.

Preparation of reagents

Binding Buffer: add specified amount of Isopropanol:

Item	100% Isopropanol to be added
48 reactions (**LSBL0048**)	18 ml
96 reactions (**LSBL0096**)	36 ml

Wash Buffer ½: add specified amount of 100% EtOH:

Item	100% EtOH to be added
NAxtra™ Wash buffer 1/2 **(LSW10048)**	42 ml
NAxtra™ Wash buffer 1/2 **(LSW10096)**	84 ml

Prepare WASH 3: Prepare enough 70% EtOH buffer to be used at wash step 3, e.g.:

Item	100% Ethanol	RNase/DNase free Water
WASH 3 (48 reactions)	24.5 ml	10.5 ml
WASH 3 (96 reactions)	49 ml	21 ml

Manual extraction of nucleic acids

Before you begin

- Ensure that you read and understand the information provided in this guide before you begin the extraction procedure.
- Review your assay documentation to determine if an extraction control is recommended to verify the efficacy of the nucleic acid preparation. Follow the extraction control guidelines provided in the assay documentation.
- Determine the number of required reactions based on the number of samples to be processed, plus appropriate positive and/or negative controls per plate.
- **Ensure that all NAxtra™ MAGNETIC BEADS are resuspended by shaking/vortexing the bottle.**
- Prepare ready to use **NAxtra BEAD MIX**: **20 µl** of the concentrated NAxtra™ MAGNETIC BEADS to **380 µl** NAxtra™ Binding Buffer per reaction, plus 10% overage.

Preparation of Bead/Binding MIX:

#Samples	Concentrated beads	Binding Buffer	Bead/Binding MIX
1	20 µl	380 µl	400 µl
48 (10% extra)	1056 µl	20064 µl	21120 µl
96 (10% extra)	2112 µl	40128 µl	42240 µl

Protocol guide for manual extraction

1. Preheat Elution buffer on **60°C**, enough for x number of samples.
2. Aliquot **10-200 µl** EDTA Whole Blood samples into a 1,5 mL tube
3. Add **315 µl** Lysis Buffer and **5 µl** Proteinase K. Mix by vortexing and incubate at **60°C for 5 min** (On shaking if possible).
4. After incubation add **400 µl** NAxtra™ Bead/Binding MIX. Mix by vortexing or pipetting several times (at least 10 times) and leave at room temperature with shaking (900 rpm) for **2 min**.
5. Place on a magnetic rack and wait until the liquid is clear (2–5 min). Remove and discard supernatant without disturbing the bead pellet.
6. Resuspend and wash the beads in **600 µl WASH 1/2** and shake for **2 min** (900 rpm).
7. Place on a magnetic rack and wait until the liquid is clear (2–5 min). Remove and discard supernatant without disturbing the bead pellet.
8. Resuspend and wash the beads in **600 µl WASH 1/2** and shake for **2 min** (900 rpm).
9. Place on a magnetic rack and wait until the liquid is clear (2–5 min). Remove and discard supernatant without disturbing the bead pellet.
10. Resuspend and wash the beads in **600 µl WASH 3** and shake for **2 min** (900 rpm).
11. Place on a magnetic rack and wait until the liquid is clear (2–5 minutes). Remove and discard supernatant without disturbing the bead pellet.
12. Dry the beads for **10 min** at room temperature, NB! Important that the beads are dried completely.
13. Resuspend the beads in **50-200 µl** of the preheated **Elution buffer**, mix by vortexing or pipetting several times (at least 10 times) and leave at room temperature with shaking (900 rpm) for **2 min**.
14. Place on a magnetic rack and wait until the liquid is clear (2–5 min). **Transfer the clear supernatant to a new RNase/DNase-free storage tube**.

Quick guide – manual nucleic acid extraction

Read full protocol guide for detailed description of instruction for use.

Step	Action	Practical
1	Place Elution buffer on 60°C	
2	Aliquot 100 µl Whole Blood samples	•In a 1,5 mL tube
3	Add, vortex and incubate: • 315 µl Lysis Buffer • 5 µl Proteinase K solution	•Fully mix •Incubate 60°C 5 min
4	Combine: • Lysed sample • 400 µL Bead/Binding Buffer	• Mix by pipetting 5 times • Shake 900 rpm 5 minutes •Magnetize! •Remove all liquid
5	Add: • 600 µl WASH 1/2	• Mix by pipetting 5 times • Shake 900 rpm 2 minutes • Magnetize! • Remove all liquid
6	Add: • 600 µL WASH 1/2	• Mix by pipetting 5 times • Shake 900 rpm 2 minutes • Magnetize! • Remove all liquid • Air dry for 10 minutes
7	Add: • 50 – 200 µL Elution Buffer	• Shake 900 rpm 2 minutes • Magnetize! • Move liquid to a new tube/plate

Automated extraction using KingFisher™ Duo

Ensure that the KingFisher™ Duo Prime Purification System with 12 pin magnet is set up and that the correct script is loaded on to the instrument. For automation scripts and protocols visit the Documentation and support section or email to contact@lybescientific.com.

> **IMPORTANT!** Failure to use the proper magnetic head results in lower yields and potential harm to the instrument.

Sample preparation and Automated extraction

1. In **Row A** of a Deep-Well Plate aliquot **10-100 µl** Whole Blood samples.
2. Add **315 µl** Lysis Buffer and **5 µl** Proteinase K solution.
3. Place a KingFisher™ Duo 12 Tip Comb for 96 DW in **Row B**, put the plate into the KingFisher™ DUO instrument.
4. Select the **NAxtra™ Blood Lysis KFD** script on the KingFisher™ DUO system and start the run, load the prepared plate into position when prompted.
5. After the run is finished (~6 min) take the plate out of the instrument. Leave the 12 DW Tip Comb in the plate.
6. Add **400 µl** Binding-Mix (according to table below) to **Row A**.

Binding-Mix	Volume (µL) per sample	12 Samples (mL)
NAxtra™ MAGNETIC BEADS	20	0.24
NAxtra™ Binding Buffer	380	4.56

7. Prepare rest of the processing plate and elution strip according to the table below.

Row ID	Reagent/Equipmet	Plate type	Total Volume (µL)
A	Sample/Lysis/BindingMix	KingFisher™ Deep-Well Plate	820
B	WASH 1/2		600
C	WASH 1/2		600
D	WASH 3 (70% EtOH)		600
H	12-Comb		
12-strip	Elution Buffer	KingFisher™ elution strip 12 pin magnet	50 - 200

8. Select the **NAxtra™ Blood extraction DUO** script on the KingFisher™ Duo Prime Purification system and start the run, load the prepared plate into position when prompted.

9. After ~16 min the run will be completed, remove elution plate and seal with proper sealing foil. Dispose used processing plates according to local routine.

The user must validate the NAxtra™ Blood total nucleic acid extraction kit in conjunction with the automation platform and consumables used and the downstream assay. Appropriate controls (e.g. internal controls, extraction controls, positive / negative controls) should be used.

Automated extraction using KingFisher™ Flex

Ensure that the KingFisher™ Flex Purification System with 96 Deep-Well Head is set up and that the correct script is loaded on to the instrument. For automation scripts and protocols visit the Documentation and support section or email to contact@lybescientific.com.

> **IMPORTANT!** Failure to use the proper magnetic head results in lower yields and potential harm to the instrument.

Sample preparation and Automated extraction

1. Prepare a Deep-Well processing plate by aliquoting **10-100 µl** Whole Blood samples.
2. Add **315 µl** Lysis Buffer and **5 µl** Proteinase K solution in each well containing sample.
3. Select the **NAxtra™ Blood Lysis KFF** script on the KingFisher™ FLEX system and start the run, load the prepared plate into position when prompted.
4. After the run is finished (~7 min) remove the sample plate from the instrument. Keep the loading plate with the 96 DW Tip Comb for the next script.
5. Add **400 µl** Binding-Mix per sample (according to table below) in the processing plate containing the Lysed blood samples.

Binding-Mix	Volume (µL) per sample	96 samples (mL)
NAxtra™ MAGNETIC BEADS	20	1.92
NAxtra™ Binding Buffer	380	36.48

6. Prepare the rest of the processing plates and elution plate according to the table below.

Plate ID	Reagent	Plate type	Volume (µL)	Plate position
Wash 1 Plate	WASH 1/2	KingFisher™ Deep-Well Plate	600	2
Wash 2 Plate	WASH 1/2		600	3
Wash 3 Plate	WASH 3 (70% EtOH)		600	4
Elution Plate	Elution Buffer	Nunc™ 96-Well Polypropylene KingFisher™ Deep-Well Plate	50-200	5
Comb Plate	Empty plate with Comb			6

7. Select the **NAxtra™ Blood extraction KFF** script on the KingFisher™ FLEX system and start the run, load the prepared plate into position when prompted.
8. After ~17 min the run will be completed, remove elution plate and seal with proper sealing foil. Dispose used processing plates according to local routine.

The user must validate the NAxtra™ Blood total nucleic acid extraction kit in conjunction with the automation platform and consumables used and the downstream assay. Appropriate controls (e.g. internal controls, extraction controls, positive / negative controls) should be used.

Troubleshooting

Issue	Possible reason	Suggested action
Magnetic bead carry-over/ Loss of magnetic beads	Magnetic separation time too short, all magnetic beads not pelleted	Increase separation (time on magnet) time
	Magnetic bead pellet disturbed	Be careful not to disturb bead-pellet when aspirating
	Aspiration speed too high, resulting in disturbing of pellet	Aspirate at a lower speed, not disturbing the pellet
	Gel-matrix formation in eluate	Increasing elution volume
Poor yield/sensitivity	Magnetic bead pellet disturbed	Be careful not to disturb bead-pellet when aspirating
	Over-drying of bead pellet, resulting in reduced elution efficiency	Reduce drying time
	Residual wash buffer carry-over in eluate	Ethanol in eluate could reduce enzyme efficiency downstream; be careful to remove all wash buffer before next step
	Different blood stabilizers may cause inhibition problems in downstream applications.	Use the recommended EDTA blood stabilizer during sample collection
Low purity/poor performance in downstream applications	Residual wash buffer carry-over in eluate	Ethanol in eluate could reduce enzyme efficiency downstream; be careful to remove all wash buffer before next step
	Eluate could be contaminated by inhibitors	For RT-qPCR try dilution of eluate. Type of contamination can be analyzed using UV-vis spectrophotometer.

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following reference provide general guidelines when handling biological samples in laboratory environment.

- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs) www.who.int/publications/i/item/9789240011311

Documentation and support

Customer and technical support

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Lybe Scientific AS and/or its affiliate(s) warrant their products as set forth in the Lybe Scientific General Terms and Conditions of Sale.

If you have any questions, please contact Lybe Scientific at www.lybescientific.com or contact@lybescientific.com.