INSTRUCTIONS FOR USE

NAxtra™ nucleic acid extraction kit

Lybe SCIENTIFIC

NAxtra™ nucleic acid extraction kit

INSTRUCTIONS FOR USE

Catalog number: LSNX200, LSNX1000, LSNX5000, LSLY200, LSLY1000, LSLY5000, LSMB200, LSMB1000 and LSMB5000

Revision 02

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.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LYBE SCIENTIFIC AS AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Pub. No: LB00001/02

Revision	Date	Description
01	21 May 2021	New document.
02	10 May 2022	Inclusion of automation protocols.

Lybe Scientific AS
Dronningens gt. 12
N-7011 Trondheim
Norway

Contents

General information

Intended use

NAxtra™ nucleic acid extraction kit is a magnetic bead-based technology intended for RNA and DNA extraction from human nasopharyngeal and/or oropharyngeal swab samples, saliva, urine or vaginal swab samples. The product should be used for isolation and purification of bacterial or viral nucleic acids preparing clinical samples for downstream qualitative diagnostics and analysis, such as PCR and next-generation sequencing. The kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in magnetic bead based nucleic acid purification and *in vitro* diagnostic procedures.

Product information

NAxtra™ nucleic acid extraction kit (Cat. No. LSNX200, LSNX1000 and LSNX5000, or sold separately as LSLY200, LSLY1000, LSLY5000, LSMB200, LSMB1000, LSMB5000) is specifically designed to recover RNA and DNA from clinical samples. The kit is based on magnetic bead technology, ensuring sensitive and high-quality recovery of nucleic acids for down-stream applications within *in vitro* diagnostics.

Kit specifications

NAxtra™ nucleic acid extraction kit can be used by manual handling protocols as well as on common liquid handling instruments or automated magnetic separators. The actual processing time depends on the configuration of the instrument and the magnetic separation system used. Typically, 96 samples can be purified in less than 15 minutes using the NAxtra™ 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 *in vitro* diagnostic use 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 proteinase K
- No need for carrier RNA
- Elution volume of 50-100 µl

Contents and storage

The NAxtra™ nucleic acid extraction kit contains sufficient reagents for 200 (LSNX200), 1000 (LSNX1000) or 5000 (LSNX5000) reactions with 100 µl or 200 µl sample input volume. Review your assay documentation to determine optimal sample input volume.

Component	Amount (LSNX200, LSLY200 or LSMB200)	Amount (LSNX1000, LSLY1000 or LSMB1000)	Amount (LSNX5000, LSLY5000 or LSMB5000)	Storage
NAxtra™ LYSIS BUFFER	40 mL	200 mL	1000 mL	2^0C-8^0C
NAxtra™ MAGNETIC BEADS	4 mL	20 mL	100 mL	

Required materials not supplied: Manual extraction

Item	Description
100% Isopropanol	Bead mix and wash solution
80% Ethanol	Wash solution
Molecular Biology Grade Water (Nuclease-free)	Elution buffer
Plastic consumables (tubes, plates, tips)	As appropriate
Pipettes	As appropriate
Vortex	As appropriate
Magnetic stand	Separation of beads from solution

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

Item	Description
100% Isopropanol	Bead mix and wash solution
80% Ethanol	Wash solution
Molecular Biology Grade Water (Nuclease-free)	Elution buffer
KingFisher™ Flex Purification System, KingFisher 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
Pharma 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.

Materials not supplied: Suggested materials for automated extraction using TECAN Fluent® 1080

Item	Description
100% Isopropanol	Bead mix and wash solution
80% Ethanol	Wash solution
Molecular Biology Grade Water (Nuclease-free)	Elution buffer
TECAN Fluent® 1080 Automated Work Station	TECAN (Bergman Diagnostika AS), Catalog number: 30042030
Deep well plates: 96-Well Storage, Assay, and Collection Plates, 2 mL well volume	Agilent, Catalog number: 201240-100
Reservoir: Single cavity, polypropylene, 300 mL, 96 pyramids base geometry	Agilent, Catalog number: 201244-100
Elution plates: 96-Well Storage, Assay, and Collection Plates, 0.5 mL and 0.7 mL well volume	Agilent, Catalog number: 204600-100
Standard tray LiHa Disposable Tips, Filtered, 200 µl	TECAN, Catalog number: 10612553
MCA 96 Disposable Tips in ANSI/SLAS-format boxes, Filtered, 200 µl	TECAN, Catalog number: 30038618

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

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 patient samples to be processed, plus one Negative Control per plate.
- Sample input volume may be **100 µl** or **200 µl**. Sensitivity may be increased if using 200 µl sample input volume.
- Ensure that all NAxtra™ MAGNETIC BEADS are resuspended by shaking the bottle.
- Prepare ready to use **BEAD MIX**: **20 µl** of the concentrated NAxtra™ MAGNETIC BEADS to **380 µl** (100µl sample input) or **580 µl** (200 µl sample input) Isopropanol per reaction, plus 10% overage. Amount of concentrated beads is independent of sample input volume.
- Prepare fresh **80% Ethanol** using Ethanol, Absolute, Molecular Biology Grade and Nuclease-free Water (not DEPC-Treated) for the required number of reactions, plus 10% overage.

Preparation of BEAD MIX:

Sample input	Concentrated beads	Isopropanol	BEAD MIX
100 µl	20 µl	380 µl	400 µl
200 µl	20µl	580µl	600 µl

Protocol guide for manual extraction

1. Pipette out **200 µl** NAxtra™ LYSIS BUFFER (pr. sample tube or pr. well if using well plate). The volume of NAxtra™ LYSIS BUFFER should be 200 µl independently of sample volume input.
2. Add **100 µl** (or **200 µl**) patient sample, mix by pipetting several times (at least 5 times up and down) and leave at room temperature with shaking (900 rpm) for **5 min**.
3. Resuspend the ready to use NAxtra™ MAGNETIC BEADS diluted in Isopropanol. Vortex thoroughly to resuspend all beads.
4. Add **400 µl** (or **600 µl**) BEAD MIX to each sample/lysis tube and mix (pipetting/vortexing).
5. Keep the beads in solution for **10 min** by shaking (900 rpm).
6. Place on a magnetic stand and wait until the liquid is clear (2–5 min). Remove and discard supernatant without disturbing the bead pellet.
7. Resuspend and wash the beads in **400 µl** 100% Isopropanol, shaking **2 min** (900 rpm).
8. Place on a magnetic stand and wait until the liquid is clear (2–5 min). Remove and discard supernatant without disturbing the bead pellet.
9. Resuspend and wash the beads in **400 µl** 80% EtOH, shaking **2 min** (900 rpm).
10. Place on a magnetic stand and wait until the liquid is clear (2–5 minutes). Remove and discard supernatant without disturbing the bead pellet.
11. Resuspend and wash the beads in **400 µl** 80% EtOH, shaking **2 min** (900 rpm).
12. Place on a magnetic stand and wait until the liquid is clear (2–5 min). Remove and discard supernatant without disturbing the bead pellet.
13. Dry the beads for **10 min** at room temperature, NB! Important that the beads are dried completely.
14. Resuspend the beads in **50 µl** Elution buffer, shaking **5 min** (900 rpm).
15. Place on a magnetic stand 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	Combine: • 200 µL Lysis Buffer • 100 µl or 200 µL Sample	• Mix by pipetting 5 times • Wait 5 minutes
2	Add: • 400 µl or 600 µL BEAD MIX	• Mix by pipetting 5 times • Shake 900 rpm 10 minutes • Magnetize! • Remove all liquid
3	Add: • 400 µL isopropanol	• Shake 900 rpm 2 minutes • Magnetize! • Remove all liquid
4	Add: • 400 µL 80% EtOH	• Shake 900 rpm 2 minutes • Magnetize! • Remove all liquid
5	Add: • 400 µL 80% EtOH	• Shake 900 rpm 2 minutes • Magnetize! • Remove all liquid • Air dry for 10 minutes
6	Add: • 50 µL Elution Buffer	• Shake 900 rpm 5 minutes • Magnetize! • Move liquid to a new tube/plate

Automated extraction using KingFisher™ Flex

1. 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.
2. Prepare the processing plates according to the table below. Plates may be prepared in advance, sealed with proper foil seal, and stored at 4°C <2 weeks.

Plate ID	Reagent	Plate type	Volume (µL)	Plate position
Wash 1 Plate	Isopropanol	KingFisher™ Deep-Well Plate	400	2
Wash 2 Plate	80% Ethanol		400	3
Wash 3 Plate	80% Ethanol		400	4
Elution Plate	Water	Nunc™ 96-Well Polypropylene Storage Microplates	50	5
Comb Plate	Empty plate with Comb			6

3. Shake/vortex the magnetic beads to ensure homogeneous suspension.
4. Prepare NAxtra™ magnetic bead mix times the number of samples to be extracted:

Reagent	Volume (µL)
NAxtra™ MAGNETIC BEADS	20
Isopropanol	380
Total volume	400

5. Prepare Lysis plate by first adding **200 µL** NAxtra™ Lysis Buffer to each well to be extracted, and then add **100 µL** or **200 µl** sample. Mix by pipetting at least 5 times and incubate for 5 min at RT.
6. Add **400 µL** or **600 µl** of NAxtra™ magnetic bead mix to each well to be extracted.

7. Select the correct script to run the NAxtra™ nucleic acid extraction kit protocol on the KingFisher™ Flex system and start the run, then load the prepared plates into correct position when prompted by the instrument.
8. After ~15 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™ nucleic acid extraction kit in conjunction with the automation platform and consumables used and the downstream in-vitro diagnostic assay. Appropriate controls (e.g. internal controls, extraction controls, positive / negative controls) should be used.

Automated extraction using TECAN Fluent® 1080 Automated Work Station

1. Ensure that the TECAN Fluent®1080 Automated Work Station is set up and that the correct file is loaded on to the instrument. For automation scripts and protocols visit the Documentation and support section or email to contact@lybescientific.com. **IMPORTANT!** Ensure that the instrument is intact and appropriately calibrated before start.
2. Shake/vortex the magnetic beads to ensure homogeneous suspension.
3. Prepare NAxtra™ BEADMIX times the number of samples to be extracted:

Reagent	Volume (µL)
NAxtra™ MAGNETIC BEADS	20
Isopropanol	380
Total volume	400

4. Prepare the Tecan Fluent®1080 Automated Work Station and select the appropriate script on the Tecan Fluent® 1080 system. Start the run, and load the prepared tips, plates and reservoirs containing NAxtra™ LYSIS BUFFER, NAxtra™ BEADMIX, Isopropanol, Ethanol and Elution Buffer according to the layout prompted in the script.
5. After approximately 45 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™ nucleic acid extraction kit in conjunction with the automation platform and consumables used and the downstream in-vitro diagnostic 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
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
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.
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

Visit www.lybescientific.com or e-mail contact@lybescientific.com for the latest service and support information.

- Worldwide contact telephone numbers
  - Product support information
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - 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