ClassiX DNA/RNA

(REF: CXDR-96)

Instructions for Use

Content

Intended use

ClassiX DNA/RNA is intended to be used for manual and automated extraction and purification of DNA and RNA from various biological samples.

Principles of the method

ClassiX DNA/RNA employs magnetic particle technology to purify nucleic acids. This approach merges the rapid, efficient performance of silica-based purification with the user-friendly handling of magnetic beads.

The workflow is designed to offer safe, consistent processing of potentially infectious material. Mixing the sample material with Buffer XL inactivates pathogens and stops nuclease activity. Proteinase K further facilitates cell disruption, nucleic acid release, and irreversible nuclease inactivation.

Subsequently, released nucleic acids are bound to Beads D in the presence of Buffer XB. Further wash steps utilizing Buffer XW1 and Buffer XW2 remove cell debris, protein background, and other sample matrix components, which lead to highly purified nucleic acids. Remaining ethanol is then removed by an air-drying step. Finally, RNA and DNA are eluted from magnetic particles using Buffer XE. A general recovery yield cannot be provided, because it varies largely with the sample material being processed.

Content of the kit

Buffer XL
Proteinase K
Beads D
Buffer XE
Buffer XB (provided as concentrate)
Buffer XW1 (provided as concentrate)
Buffer XW2 (provided as concentrate)

Programs for automation

Use the SwiftXtractor™ SL protocol CXDR-SXT200.

The program files and program sheets are available on request: contact our technical support via sales@xpedite-dx.com.

Equipment to be provided by the user (manual process)

- Appropriate personal protective equipment
- Pipets and disposable pipet tips (aerosol barriers recommended)
- 1.5 mL microcentrifuge tubes
- Magnetic stand (Xpedite Diagnostics REF: MAG-12, MAG-4)
- Vortexer (Xpedite Diagnostics REF: VOR-01)
- Heating block (Xpedite Diagnostics REF: ACC-15)
- Mini spin centrifuge (Xpedite Diagnostics REF: CEN-01)
- 96% (v/v) or absolute ethanol (do not use denatured ethanol)
- Isopropanol

Equipment to be provided by the user (automated process)

- Appropriate personal protective equipment
- Pipets and disposable pipet tips (aerosol barriers recommended)
- SwiftXtractor™ (Xpedite Diagnostics REF: SXT-SL)
- SwiftXtractor rod sleeves (Xpedite Diagnostics REF: SXT-RS-100)
- 2 mL microcentrifuge tubes (Xpedite Diagnostics REF: SXT-2mL-250)
- 5mL tubes (Xpedite Diagnostics REF: SXT-5mL-250, SXT-5mLC-500)
- Optional: SwiftXtractor Sample Preparation Rack (REF: SXT-R)
- 96% (v/v) or absolute ethanol (do not use denatured ethanol)
- Isopropanol

Storage and shelf life

ClassiX™ DNA/RNA kits must be stored at 15 °C to 25 °C. Storage at lower temperatures leads to formation of salt crystals. Reagents are good to be used until the expiry date indicated on the label. Do not use reagents after their indicated expiry date.

After first use, ClassiX™ DNA/RNA kits are good to be used within 3 months.

Warnings and precautions

ClassiX™ DNA/RNA comprises several components with hazardous substances. The Safety Data Sheet (SDS) is available upon request. The following hazard and precaution statements apply:

Buffer XL and Buffer XW1:

Danger

Contain guanidinium hydrochloride

H302	Harmful if swallowed
H332	Harmful if Inhaled
H315	Causes skin irritation.
H319	Causes serious eye irritation.
P271	Use only outdoors or with adequate ventilation.
P280	Wear protective gloves, eyes, and face protection.
P302	Harmful if swallowed or in contact with skin or if inhaled.
P362	Take off contaminated clothing.
P501	Dispose of contents/container in accordance with all regional, national, and international regulations.

Buffer XB:

Danger

Contains guanidinium thiocyanate

H302	Harmful if swallowed
H313+H332+H333	May be harmful in contact with skin or if inhaled.
H314	Causes severe skin burns and eye damage.
H412	Harmful to aquatic life with long lasting effects.
EUH032	Contact with acids liberates very toxic gas.
P280	Wear protective gloves, eyes, and face protection.
P273	Avoid release to the environment.
P303+P361+P353	IF ON SKIN/HAIR: Take off contaminated clothing. Rinse with water.
P305+P351+P338	IF IN EYES: Rinse continuously with water for 5 minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P308+P310	IF exposed or concerned: Immediately call a POISON CENTER or doctor/physician.
P330	Rinse mouth.
P331	Do NOT induce vomiting.
P340	Remove person to fresh air and keep comfortable for breathing.
P361	Take off immediately all contaminated clothing.
P363	Wash contaminated clothing before reusing.
P501	Dispose of contents/container in accordance with all regional, national, and international regulations.

Proteinase K:

Danger

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P264+P352	Wash respective body parts after accidental contact.
P280	Wear protective gloves, eyes, and face protection.
P302	Harmful if swallowed or in contact with skin or if inhaled.
P332+P313	If skin irritation occurs: get medical advice/attention.
P362+P364	Take off contaminated clothing and wash it before reusing.
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P313+P337	If eye irritation persists: Get medical advice/attention.
P501	Dispose of contents/container in accordance with all regional, national, and international regulations.

Buffers XL, Buffer XW1, and Buffer XB contain guanidinium salts, which can form highly reactive compounds if mixed with bleach. If liquid containing these buffers is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, disinfect with alcoholic disinfectants but not with bleach.

Take care when working with biological samples and always treat them as potentially infectious. Users are advised to always wear appropriate personal protective equipment.

Nucleic acid extracts can be disposed of with regular laboratory waste. Please take your national regulations for waste sorting and treatment into consideration.

Make sure to work with clean equipment and use pipette tips with aerosol barriers to avoid carryover of specimens or nucleic acid extracts between samples.

Preparation of reagents before use

Buffer XB:

Buffer XB is supplied as a concentrate and must be mixed with the volume of isopropanol indicated on the lid of the bottle before it can be used. Tick the check box on the lid label of the bottle to indicate that isopropanol has been added. Mix well before use. To avoid evaporation, keep bottle closed if not in use.

Beads D:

Shake or vortex Beads D for 3 min before first use to ensure that the magnetic particle suspension is homogeneous.

Buffer XW1:

Buffer XW1 is supplied as a concentrate and must be mixed with the volume of 96% (v/v) or absolute ethanol indicated on the lid of the bottle before it can be used. Do not use denatured ethanol. Tick the check box on the lid label of the bottle to indicate that ethanol has been added. Mix well before use. Keep bottle closed if not in use.

Buffer XW2:

Buffer XW2 is supplied as a concentrate and must be mixed with the volume of 96% (v/v) or absolute ethanol indicated on the lid of the bottle before it can be used. Do not use denatured ethanol. Tick the check box on the lid label of the bottle to indicate that ethanol has been added. Mix well before use. Keep bottle closed if not in use.

Recommended third wash

A third wash step with 96% (v/v) or absolute ethanol is recommended. Do not use denatured ethanol. Use the volume as described in the respective protocol below.

For automation process:

Reagents can be prepared beforehand in the SwiftXtractor™ preparation Rack (REF: SXT-R) as described in detail in section “Protocol for automated extraction of DNA and RNA on SwiftXtractor”. For short-term storage: Tubes can be stored with screw-caps on until use for extraction. Before starting the extraction process, transfer all the tubes in the right order to the SwiftXtractor™.

Note: Remove the lids before starting the extraction process.

Protocol for manual extraction and purification of DNA and RNA

To do before starting

- **Read the complete protocol.**
- Set the heating block to 65 °C.
- Shake or vortex Beads D for 30 seconds to homogenize the particle suspension.
- Make sure Buffer XB, Buffer XW1, and Buffer XW2 have been mixed with isopropanol and ethanol, respectively (see section "Preparation of reagents before use").

Please note: The protocol can be shortened by combining lysis of material (Steps 1 - 4) and binding of nucleic acids (Steps 5 - 6). See section "Protocol for combined cell lysis and nucleic acid binding" for detailed instructions.

1. **Add 200 µL sample to a 1.5 mL microtube.** If the sample volume is less than 200 µl, add PBS to bring the total volume to 200 µl.

2. **Add 20 µL of Proteinase K and mix well by vortexing for 5 seconds. Ensure that no liquid is resting at the tube lid.**

3. **Add 100 µL of Buffer XL and mix well by vortexing for 5 seconds. Ensure that no liquid is resting at the tube lid.**

4. **Incubate microtube at 65 °C for 10 minutes. Samples can be shaken at 700 rpm if a thermo-shaker is used.**

5. **Add 400 µL Buffer XB and 25 µL Beads D and mix well by vortexing for 30 seconds. Ensure that no liquid is sitting at the tube lid. Spin the tube briefly if necessary.**

6. **Incubate microtube at room temperature for 10 minutes.**

7. **Place microtube in a magnetic stand for 2 minutes.**

8. **Open microtube while remaining in the magnetic stand. Remove and discard the supernatant by pipetting.**

9. **Add 700 µL of Buffer XW1 and mix well by vortexing for 15 seconds. Ensure that no liquid is sitting at the tube lid. Spin the tube briefly if necessary.**

10. **Place microtube in a magnetic stand for 1 minute.**

11. **Open the microtube while remaining in the magnetic stand. Remove and discard the supernatant by pipetting.**

12. **Add 700 µL of Buffer XW2 and mix well by vortexing for 15 seconds. Ensure that no liquid is sitting at the tube lid. Spin the tube briefly if necessary.**

13. **Place microtube in a magnetic stand for 1 minute.**

14. **Open the microtube while remaining in the magnetic stand. Remove and discard the supernatant by pipetting.**

15. **<u>Optional</u>: Repeat steps 12 to 14 with 750 µL of 96% non-denaturated ethanol as a third wash step.**

16. **Incubate the opened microtube at room temperature for 10 minutes while remaining in the magnetic stand to evaporate remaining wash buffer or ethanol.**

17. **Add 50 µL to 200 µL Buffer XE and mix well by vortexing for 5 seconds.**

18. **Incubate microtube at 65 °C for 5 minutes. Samples can be shaken at 700 rpm if a thermo-shaker is used.**

19. **Vortex the microtube again for 5 seconds. Ensure that no liquid is sitting at the tube lid. Spin the tube briefly if necessary.**

20. **Place microtube in a magnetic stand for 30 seconds.**

21. **Open the microtube while remaining in the magnetic stand. Transfer supernatant to a new microtube.**

Purified DNA can be stored for up to 7 days at 4 - 8 °C or up to 4 weeks at -20 °C. Purified RNA can be stored for up to 24 hours at 4 - 8 °C or up to 3 days at -30 °C to -15°C. For long-term storage, RNA should be stored -80 °C. Multiple freeze-thaw cycles and long storage time may cause degradation.

Always mix samples before downstream analysis if they have been stored.

Protocol for combined cell lysis and nucleic acid binding (manual process)

To do before starting

- **Read the complete protocol.**
- Set the heating block to 65 °C.
- Shake or vortex Beads D for 30 seconds to homogenize the particle suspension.
- Make sure Buffer XB, Buffer XW1, and Buffer XW2 have been mixed with isopropanol and ethanol, respectively (see section “Preparation of reagents before use”).

1. **Add 20 µL Proteinase K to a 1.5 mL microtube and add 200 µL of sample. Mix well by vortexing for 5 seconds.** If the sample volume is less than 200 µl, add PBS to bring the total volume to 200 µl.

2. **Prepare a mix of 100 µL Buffer XL, 400µL Buffer XB, and 25µL Beads D and mix well by vortexing for 10 seconds. Ensure that no liquid is resting at the tube lid.**

3. **Add the reagent mix prepared in Step 2 to the sample tube and mix well by vortexing for 10 seconds.**

4. **Incubate microtube at 65 °C for 5 minutes. Samples can be shaken at 700 rpm if a thermo-shaker is used.**

5. **Continue with Step 7 in section “Step-by-step protocol for extraction and purification of DNA and RNA”.**

Purified DNA can be stored for up to 7 days at 4 - 8 °C or up to 4 weeks at -20 °C.Purified RNA can be stored for up to 24 hours at 4 - 8 °C or up to 3 days at -30 °C to -15°C. For long-term storage, RNA should be stored -80 °C. Multiple freeze-thaw cycles and long storage time may cause degradation.

Always mix samples before downstream analysis if they have been stored.

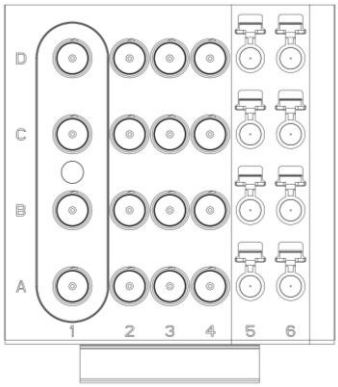
Protocol for automated extraction of DNA and RNA on SwiftXtractor™

Ensure that you are familiar with the correct operation of the workstation. Refer to the respective user manual for operating instructions. Make sure Buffer XB, Buffer XW1, and Buffer XW2 have been mixed with isopropanol and ethanol, respectively (see section "Preparation of reagents before use").

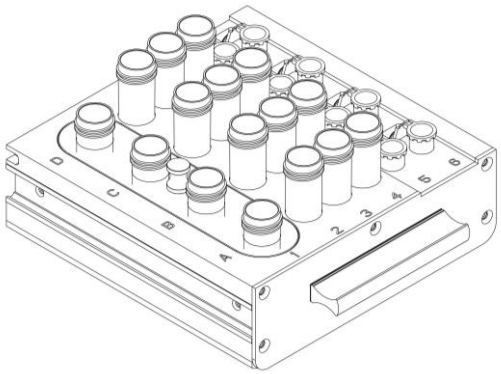
Reagent preparation for performing **CXDR-SXT200** on SwiftXtractor™ SL:

Note: 4 samples can be processed at a time (labeled A-D)

A:



B:



1. Insert the 5 mL adaptor in position 1
2. Prepare **sample tube:** Add **20µl** of **Proteinase K** to **200µl of sample** in 5 mL tubes (and insert in position 1)
3. Prepare **Lysis mix**: Add **100µl Buffer XL, 400µl Buffer XB and 25µl Beads D per sample** in a tube.

Note: Prepare sufficient mixture volumes for the total amount of samples planned to be extracted.

4. Prepare **Wash tubes** 1: Add **1 mL of Buffer XW1** into 5 mL tubes (and insert in position 2)
5. Prepare **Wash tubes** 2: Add **1 mL of Buffer XW2** into 5 mL tubes (and insert in position 3)
6. Prepare **Wash tubes** 3: Add **1 mL of 96-100% non-denatured Ethanol** into 5 mL tubes (and insert in position 4)
7. Add required amounts of "**rod sleeves**" to 2 mL microtubes (and insert into position 5)
8. Add **100 µl Buffer XE** to 2 mL tubes (and insert in position 6)
9. Add **525 µL of Lysis mix (from step 3)** into **sample tube**.
10. Start appropriate protocol.

Overview of buffer volumes needed (per reaction):

Position	**1**	**2**	**3**	**4**	**5**	**6**
Step	**Lysis**	**Wash 1**	**Wash 2**	**Wash 3**		**Elute**
Item to add	Sample + Lysis mix	Buffer XW1	Buffer XW2	96-100% Ethanol	Rod sleeve	Buffer XE
Volume/Quantity	745 µl	1 mL	1 mL	1 mL	1 piece	100 µl

Purified DNA can be stored for up to 7 days at 4 - 8 °C or up to 4 weeks at -20 °C. Purified RNA can be stored for up to 24 hours at 4 - 8 °C or up to 3 days at -30 °C to -15°C. For long-term storage, RNA should be stored -80 °C. Multiple freeze-thaw cycles and long storage time may cause degradation.

Always mix samples before downstream analysis if they have been stored.

Troubleshooting guide

Observation	Likely caused by	Recommendation
No or weak PCR signals	**a) Low amount of target nucleic acid** Low pathogen/cell load in the original sample **b) Nucleic acid loss during extraction** Beads D not properly resuspended before use Incorrect preparation of Buffer XB, XW1, or XW2 Incorrect volumes of lysis mix, wash buffers, or elution buffer Elution volume too high, causing dilution of the nucleic acid **c) Nucleic acid degradation** Incorrect storage temperature after extraction Prolonged storage or repeated freeze–thaw cycles	If the original sample contains only a low pathogen load, concentrate the sample before extraction. If weak signals persist, repeat extraction from a fresh sample. Ensure correct sample input and reagent volumes, thoroughly resuspend Beads D before use. Verify that Buffer XB, XW1, and XW2 were prepared correctly. Store samples at appropriate temperatures. Repeat PCR with appropriate positive, negative, and internal controls.
High background signals in qPCR	Bead carryover	Avoid disturbing the particle pellet when transferring extracted DNA to a new tube. Magnetic-particle carryover can be minimized by placing the microplate containing eluates in a suitable magnet (e.g., MAG-12) for 1 min, and transfer of the eluates to a clean tube. If a suitable magnet is not available, centrifuge the tubes containing eluates at full speed for 1 min to pellet any remaining magnetic particles, and transfer the supernatants to a clean tube.

Key to symbols

	Catalog number
	Number of extractions
	Storage temperature
	Batch number
	Expiry date
	Read Instructions for Use
	Legal manufacturer

Legal manufacturer

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