

SwiftX™ Sepsis

(REF: SXFB-20)

Instructions for Use

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Intended use

SwiftX™ Sepsis is intended to be used for extraction and purification of DNA of bacterial and fungal cells as well as of leukocytes from up to 10 mL of whole blood. For research use only.

The extracted DNA can be applied to a wide range of analytical methods, such as spectrophotometric and fluorometric analyses, real-time quantitative PCR, and isothermal amplification.

Principles of the method

SwiftX™ Sepsis is designed for extraction and purification of DNA from up to 10mL of venous whole blood. The different components of SwiftX™ Sepsis have specific functionalities within the workflow of DNA extraction.

Buffer FBC stabilizes biological cells such as bacteria, fungal cells, and white blood cells during cell capturing and enables efficient binding of preferentially bacterial and fungal cells to Beads A.

Incubation with **Buffer FBW** facilitates the removal of contaminants, impurities, and inhibitors.

Buffer FBL enables an efficient cell lysis and release of DNA. Optimal cell lysis is achieved by utilizing **Proteinase K** and applying a two-step heat incubation.

Beads A are paramagnetic particles with fast and strong magnetic response utilized for binding cells and for removal of cell debris as well as other particulate matter from the lysis mixture.

Content of the kit

Buffer FBC (Cell capture buffer)	Beads A (paramagnetic particles)
Buffer FBW (Cell wash buffer)	Proteinase K
Buffer FBL (Cell lysis buffer)	

Equipment to be provided by the user

For performing the nucleic acid extraction procedure, the following laboratory equipment is required and needs to be provided by the user:

- Appropriate personal protective equipment
- Pipets and disposable pipet tips (aerosol barriers recommended)
- Sample tubes with volume >15 mL, e.g. 50 mL centrifuge tubes with screw-cap
- 2 mL and 1.5 mL microcentrifuge tubes (safe-lock-caps or screw-caps recommended)
- Magnetic stands (Xpedite Diagnostics REF: MAG-2x50mL-LV, MAG-12)
- Vortexer (Xpedite Diagnostics REF: VOR-01)
- Heating block (ideally 2 blocks are used, Xpedite Diagnostics REF: ACC-15)
- Mini spin centrifuge (Xpedite Diagnostics REF: CEN-01)

Storage and shelf life

SwiftX™ Sepsis kits must be stored at 2 °C to 8 °C. Do not freeze. 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, SwiftX™ Sepsis kits are good to be used within 3 months.

Warnings and precautions

SwiftX™ Sepsis comprises of 3 buffers and paramagnetic particles. All these components are free of hazardous substances. The Proteinase K comprised in the kit is considered an irritating substance. The safety data sheet (SDS) for SwiftX™ Sepsis is available upon request. The following hazard and precaution statements apply:

Proteinase K:

Danger.



H315	Causes skin irritation.
H319	Causes serious eye irritation.
P264	Wash respective body parts after accidental contact.
P280	Wear protective gloves, eyes, and face protection.

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.

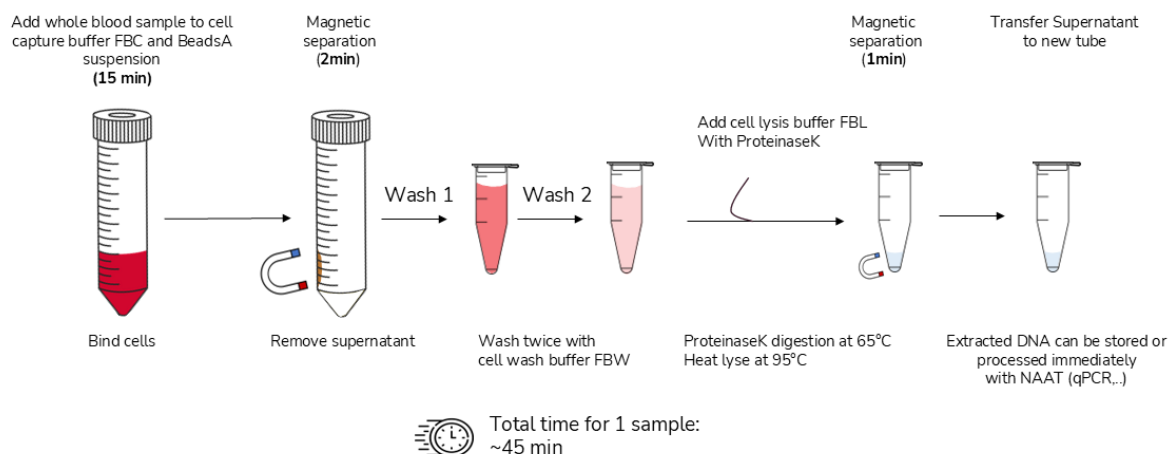
Sample types and their collection, handling, and storage

Venous whole blood should be collected using sterile equipment according to established phlebotomy procedures. SwiftX™ Sepsis was **validated for venous whole blood using Na₃ Citrate as anticoagulant. K₂ EDTA can also be used**, while **Lithium Heparin must not be used** as a blood coagulant. Blood samples should be processed without delay and kept refrigerated to avoid degradation processes in the sample. Do not freeze the blood sample as this can lead to cell rupture and, thus, to reduced extraction performance.

The optimal **sample input volume** is 10 mL of venous whole blood anti-coagulated with Na₃ Citrate. However, smaller volumes as low as 400 µL of whole blood can also be processed.

The standard **lysis volume** is 80 µL.

Graphical representation of the extraction procedure



Read before first use of the extraction kit

SwiftX™ Sepsis is optimized for extraction of cells from whole blood samples anticoagulated with citrate or EDTA. **Other sample types must be validated by the user** because they may behave differently due to their difference in chemical properties such as salinity, pH, and type of metabolic substances.

SwiftX™ Sepsis works very differently than conventional blood extraction products. Thus, first-time users may need extra attention to fully leverage the potential of the kit. The following information will help you to become aware of the most critical points of the extraction process:

- Cell capturing performance and removal of unspecific material depends on the quality of your magnetic stand. It is highly recommended to use magnetic racks with strong Neodym-magnets.
- For processing 10mL blood in 50mL tubes, the use of our MAG-2x50mL-LV racks is highly recommended. They feature optimized magnet positions leading to superior separation of particles over a large surface, which is important for removal of inhibitory substances.
- It is of utmost importance to remove supernatants completely. For that, wait several seconds after removal of the supernatant for additional liquid to drain down from the tube wall and remove this residual liquid before proceeding with the next protocol step.
- Samples must be warmed to room temperature (20-30°C) before extraction. Processing of cooled samples leads to reduced cell-capture efficiency.
- If you work with spiked samples, it is particularly important to mix the blood by vortexing and letting the spiked sample rest for 15 minutes to ensure a homogeneous distribution of spiked cells within the sample.
- Old blood samples or otherwise hemolyzed samples must be processed with great attention. Captured cells must be washed thoroughly. Complete removal of supernatants after particle separation is crucial.
- When extracting 10mL of whole blood, the DNA concentration will be approximately 200 ng/μL showing an A260/A280 ratio of 0.7 to 1.0. These values are not impacting the PCR reaction.
- Depending on the age and condition of the blood, the DNA eluate may be colored. In most cases, this does not affect PCR performance.

Step-by-step protocol for extraction of DNA from 10 mL whole blood (recommended)

Important things to do before starting:

- **It is of utmost importance to be aware of critical protocol points before starting. Please read section [“Read before first use of the extraction kit”](#) before you start the bench work!**
- Bring samples to room temperature. Cold samples show a reduced cell capturing efficiency.
- Shake or vortex Beads A for 30 seconds to homogenize the particle suspension.
- Set the heating block to 65 °C. If a second heating block can be used, then set it to 95°C.

1. Add 60 µL of Beads A and 6 mL of Buffer FBC to a 50mL tube.
2. Add 10 mL of whole blood and close the tube. Mix well by vortexing for 10 seconds or pipetting up and down. Ensure that no liquid is resting at the tube lid.
3. Incubate the sample tube at room temperature for 15 minutes.
4. Place the tube in a magnetic stand for 2 minutes.
5. Open the tube while remaining in the magnetic stand. Remove the supernatant by pouring or pipetting and discard.
6. Add 1 mL of Buffer FBW and rinse the Beads A from the tube wall to the bottom. Then transfer the bead suspension to a new 2 mL microtube and close it.
7. Mix well by vortexing for 5 seconds. Ensure that no liquid is sitting at the tube lid. Spin the tube briefly if necessary.
8. Place the tube in a magnetic stand for 1 minute.
9. Open the tube while remaining in the magnetic stand. Completely remove the supernatant by pipetting and discard. Make sure no residual liquid is left in the tube.
10. Add 1 mL of Buffer FBW and mix well by pipetting by vortexing for 5 seconds. Ensure that no liquid is sitting at the tube lid. Spin the tube briefly if necessary.
11. Place the tube in a magnetic stand for 1 minute.
12. Open the tube while remaining in the magnetic stand. Completely remove the supernatant by pipetting and discard. Make sure no residual liquid is left in the tube.
13. Add 76 µL of Buffer FBL and 4µL Proteinase K to the tube and resuspend the Beads A by vortexing for 5 seconds. Ensure all liquid is collected at the bottom of the tube.
14. Incubate the tube in a heating block at 65 °C for 10 minutes.
15. Incubate the tube in a heating block at 95 °C for 12 minutes.
16. Remove the tube from the heating block. Ensure that no liquid is sitting at the tube lid. Spin the tube briefly if necessary.
17. Place the tube in a magnetic stand for 1 minute.
18. Open tube while remaining in the magnetic stand. Transfer supernatant to a new microtube.

Please consult the section [“Troubleshooting guide”](#) if you face any issues throughout the process or with the extracted DNA.

Step-by-step protocol for extraction of DNA from 400 µL to 1 mL of whole blood

Important things to do before starting:

- **It is of utmost importance to be aware of critical protocol points before starting. Please read section [“Read before first use of the extraction kit”](#) before you start the bench work!**
 - Bring samples to room temperature. Cold samples show a reduced cell capturing efficiency.
 - Shake or vortex Beads A for 30 seconds to homogenize the particle suspension.
 - Set the heating block to 65 °C. If a second heating block can be used, then set it to 95°C.
1. Add 30 µL of Beads A and 0.6 volume of the desired sample volume of Buffer FBC (e.g. 0.6 mL Buffer FBC for 1 mL whole blood) to a 2 mL tube, respectively.
 2. Add the desired volume of whole blood (400µl – 1mL) and close the tube. Mix well by vortexing for 10 seconds or pipetting up and down. Ensure that no liquid is resting at the tube lid.
 3. Incubate the sample tube at room temperature for 15 minutes.
 4. Place the tube in a magnetic stand for 2 minutes.
 5. Open the tube while remaining in the magnetic stand. Remove the supernatant by pipetting and discard.
 6. Add 1 mL of Buffer FBW and mix well by vortexing for 5 seconds. Ensure that no liquid is sitting at the tube lid. Spin the tube briefly if necessary.
 7. Place the tube in a magnetic stand for 1 minute.
 8. Open the tube while remaining in the magnetic stand. Completely remove the supernatant by pipetting and discard. Make sure no residual liquid is left in the tube.
 9. Add 1 mL of Buffer FBW and mix well by pipetting by vortexing for 5 seconds. Ensure that no liquid is sitting at the tube lid. Spin the tube briefly if necessary.
 10. Place the tube in a magnetic stand for 1 minute.
 11. Open the tube while remaining in the magnetic stand. Completely remove the supernatant by pipetting and discard. Make sure no residual liquid is left in the tube.
 12. Add 76 µL of Buffer FBL and 4µL Proteinase K to the tube and resuspend the Beads A by vortexing for 5 seconds. Ensure all liquid is collected at the bottom of the tube.
 13. Incubate the tube in a heating block at 65 °C for 10 minutes.
 14. Incubate the tube in a heating block at 95 °C for 12 minutes.
 15. Remove the tube from the heating block. Ensure that no liquid is sitting at the tube lid. Spin the tube briefly if necessary.
 16. Place the tube in a magnetic stand for 1 minute.
 17. Open tube while remaining in the magnetic stand. Transfer supernatant to a new microtube.








Please consult the section [“Troubleshooting guide”](#) if you face any issues throughout the process or with the extracted DNA.

Troubleshooting guide

SwiftX™ Sepsis works differently than conventional blood extraction products. Thus, first-time users may face difficulties in achieving optimal results with the kit. The information below shall support you in finding solutions in case something goes wrong.

Observation	Likely caused by	Recommendation
No or weak PCR signals	<p>(a) Insufficient cell capture due to using cold blood samples</p> <p>(b) inhibitory substances in DNA eluate due to hemolysis or incomplete removal of liquids after capture and wash steps</p> <p>(c) Nanodrop and Qubit measurements are not specific for bacterial DNA but will quantify total DNA content in your sample, which is mostly from leukocytes.</p>	<p>In case of (a): ensure to process samples only if equilibrated to 20-30°C</p> <p>In case of (b): completely remove residual liquid after capturing and washing steps.</p> <p>In case of (c): do not dilute extracted DNA based on total DNA concentration, because it is not indicative for the concentration of bacterial or fungal DNA.</p>
Shift in observed Ct values against expected Ct values	Inhibitor carryover	Pay attention to the complete removal of residual liquids after cell capture- and wash steps. You may also wash a third time.
High background signals in qPCR	Bead carryover or use of a magnetic rack with strong local magnetic field	Avoid disturbing the particle pellet when transferring extracted DNA to a new tube. Use a „wide field“ magnetic rack.
Beads are clumping during or after wash steps	Extended incubation time of sample on a magnet rack after removal of liquids or use of a magnetic rack with strong local magnetic field	Try to dissolve the clumps by flicking of the tube or addition of a small buffer volume before resuspension of Beads A. If the procedure is performed manually, it is recommended to process less samples simultaneously in to minimize resting times between protocol steps.
Low reproducibility of results	Process variations	If performed manually, the extraction procedure requires practice. A training run is recommended for familiarization with bead behavior and timing.

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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