SwiftX™ ParaBact

Handbook

Content

Storage

SwiftX™ ParaBact must be stored **at 2-25°C** and can be used before the expiry date indicated on the labels.

Product Use

For Research Use Only.

SwiftX™ ParaBact is designed for magnetic capture and sensitive and rapid DNA extraction of bacteria as well as eukaryotic cells, i.e. parasitic protozoa, helminth eggs, fungi as well as human & animal cells.

SwiftX ParaBact can be applied to solid as well as liquid specimens. The recommended protocol is dependent on the respective specimen type to be processed. Applicable liquid specimens include urine, saliva, tissue homogenates, mammalian and bacterial cell cultures, cerebrospinal fluid, guanidine-free transport media, and liquid-based cytology media, sputum, blood, and wastewater. Examples for solid samples, which can be directly subjected to the lysis procedure, are oral, nasal, skin lesion and rectal swabs as well as pre-concentrated cells.

Cell capture and extraction procedures can be performed manually as well as in an automated fashion. Automation of the nucleic acid extraction protocol is possible with a variety of robotic pipetting and handling systems due to its minimal number of work steps. Any adaptation has to be performed and validated by the user.

Safety information

SwiftX™ ParaBact comprises of 3 buffers (Buffer BPE, BPL and AD) and paramagnetic particles (Beads A). Apart from Buffer BPL, all other components of the kit are free of hazardous substances. The safety data sheets (SDS) for SwiftX™ ParaBact components are available upon request.

Buffer BPL:

Danger

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

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

Quality control

Each batch of SwiftX™ ParaBact is tested against defined specifications to ensure consistent product quality. A Certificate of Analysis can be provided upon request.

Equipment to be provided by the user

- Appropriate personal protective equipment
- Pipets and disposable pipet tips (aerosol barriers recommended)
- 1.5mL and 2mL microcentrifuge tubes (safe-lock-caps or screw-caps recommended) or a deep-well plate
- Magnetic stand, Vortexer
- Heating device (water bath, heating block, or thermo shaker)

Purchase information for kit and accessories

Item	Description	Cat.no.
SwiftX™ ParaBact (25 extractions)	Kit for DNA extraction from various specimen types and organisms	SXPB-25
Mini heat block	Compact dry heat block for incubation of 15x 1.5mL microtubes	ACC-12
Magnetic separation rack	Rack with Neodymium magnets for 12x 1.5mL or 2mL microtubes *Further magnetic racks available on our website.*	MAG-12
Buffer BPE, 25 mL	Cell capturing buffer for SwiftX™ ParaBact	BPE-25mL

Principles

SwiftX™ ParaBact is designed for rapid extraction of DNA from various biological samples. The different components of SwiftX™ ParaBact have specific functionalities within the workflow of DNA extraction.

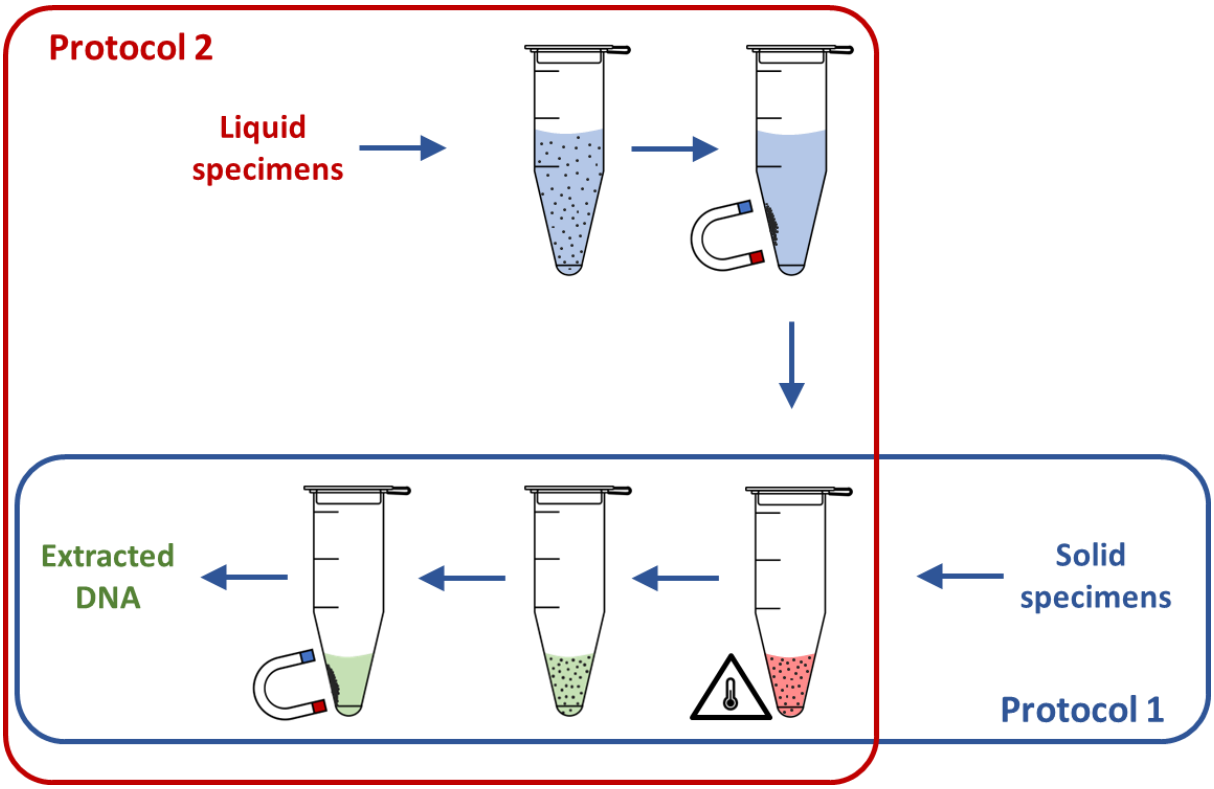
Buffer BPE stabilizes biological cells such as those from bacteria, fungi, protozoa, animal and humans during the magnetic cell capturing step and enables efficient binding especially of a wide range of bacteria to the paramagnetic particles.

The paramagnetic particles **Beads A** show a broad binding property to cells and proteins. This effect is leveraged in two ways during SwiftX™ ParaBact-based extraction. Firstly, Beads A enable a species-independent concentration of cells from liquid specimens. Secondly, during and after heat lysis, Beads A are utilized to remove cell debris and other particulate matter from the lysis mixture.

Buffer BPL enables an efficient lysis of cells (bacteria, protozoa, fungi, helminth eggs, animal & human cells), especially in conjunction with the application of heat. The buffer also actively removes RNA from the lysate.

Buffer AD is added after the cell lysis and renders the lysate containing the extracted DNA compatible with downstream applications such as PCR, isothermal amplification technologies, and nanopore sequencing.

Overview of the different workflow options



Protocol 1:

- Addition of Buffer BPL and Beads A to solid sample
- Heat incubation of the mixture
- Addition of Buffer AD to the lysate for neutralization
- Reverse purification by magnetic separation of Beads A

Protocol 2

- Addition of Buffer BPE and Beads A to liquid sample
- Incubation and magnetic separation of Beads A
- Resuspension of Beads A with Buffer BPL
- Heat incubation of the mixture
- Addition of Buffer AD to the lysate for neutralization
- Reverse purification by magnetic separation of Beads A

Protocol 3

- Same as Protocol 2, but comprising a wash step with Buffer BPE before resuspension of Beads A with Buffer BPL

Protocol 1: DNA extraction from swabs and other solid specimens

This protocol describes the workflow of extracting DNA from samples such as swabs and pre-concentrated cells. It can also be used to extract DNA from small volumes of liquid samples.

To do before starting

- ***Read the complete protocol.***
- Heat a water bath, heating block, or thermal shaker to 95°C.
- For swab samples, we recommend to elute the swabbed material with a minimum amount of saline or PBS. 50µL of swab eluate is required for extraction.

1. **Add 200µL of Buffer BPL to a microcentrifuge tube containing your sample, which can be one of the following types:**
    a. **a pellet of pre-concentrated cells**
    b. **50µL eluted swabbed sample (see "To do before starting")**
    c. **up to 50µL of liquid.**

2. **Shake or vortex Beads A for 30 seconds to ensure a homogeneous suspension. Add 30µL of Beads A to the sample mixture. Mix well for 10 seconds by vortexing or inverting the tube.**

3. **Incubate the lysis mixture at 95°C for 10 minutes.**

    Note: If a thermal shaker is used, shake at maximum speed. The heating time can range from 5 to 15 minutes and should be validated by the user for the particular application.

4. **Remove sample tube from heating device and mix well for 5 seconds. Remove the condensate from the lid before opening by shaking down or tapping the tube on the work bench.**

5. **Open the lid and add 40µL Buffer AD to the lysis mixture. Mix well for 5 seconds by vortexing or inverting the tube.**

6. **Place the sample tube into a magnetic stand at room temperature for 1 minute to let the magnetic particles separate.**

7. **Open the lid while the tube remains in the magnetic stand and transfer the supernatant into a new tube for storage or use in downstream applications.**

DNA extracts can be stored at -20°C if samples shall be processed later.

Protocol 2: DNA extraction from liquid specimens

This protocol describes the workflow of extracting DNA from up to 1mL liquid samples, such as urine, cell cultures, CSF, saliva, transport media, and wastewater. This protocol includes a cell capturing step before the DNA extraction.

To do before starting

- ***Read the complete protocol.***
- Heat a water bath, heating block, or thermal shaker to 95°C.
- Shake or vortex Beads A for 30 seconds to ensure a homogeneous suspension.
- Prepare a sufficient amount of **"capture mix"** of Buffer BPE and Beads A for your extractions as follows:
    - Determine the volume of sample you want to process (200µL – 1mL)
    - Pipette an equal volume of Buffer BPE into a tube, e.g. 500µL of Buffer BPE if you want to process 500µL of liquid sample)
    - Add 30µL of Beads A to the Buffer BPE
      Note: Do not modify the volume of Beads A! It is always 30µL - independent of the volume of Buffer BPE.
    - Mix well for 10 seconds by vortexing or inverting the tube.

1. **Add the prepared "capture mix" (see above) to a microcentrifuge tube.**

2. **Add the desired volume of liquid sample (200µL – 1mL) to the "capture mix". Mix well by pipetting up and down or by vortexing or inverting the tube for 10 seconds.**

   Note: If highly viscous samples are used, it is recommended to vortex them for 30 seconds at high speed before use in order to reduce the viscosity.

3. **Incubate the sample tube at room temperature for 3 minutes.**

   Note: If a maximum sensitivity is desired, the incubation time for cell binding can be extended up to 10 minutes.

4. **Mix well for 5 seconds, then place the sample tube into a magnetic stand and let the magnetic particles separate at room temperature for 1 minute.**

5. **Open the lid while the tube remains in the magnetic stand. Remove the supernatant completely by pipetting. Discard the supernatant.**

6. **Add 100µL of Buffer BPL to the tube and remove the tube from the magnetic stand. Resuspend the magnetic particles by vortexing or shaking for 10 seconds.**

7. **Incubate the lysis mixture at 95°C for 10 minutes.**

Note: If a thermal shaker is used, shake at maximum speed. The heating time can range from 5 to 15 minutes and should be validated by the user for the particular application.

8. **Remove the sample tube from the heating device and mix well for 5 seconds. Remove the condensate from the lid before opening by shaking down or tapping the tube on the work bench.**

9. **Open the lid and add 20µL Buffer AD to the lysis mixture. Mix well for 5 seconds by vortexing or inverting the tube.**

10. **Place the sample tube into a magnetic stand and let the magnetic particles separate at room temperature for 1 minute.**

11. **Open the lid while the tube remains in the magnetic stand and transfer the supernatant into a new tube for storage or use in downstream applications.**

DNA extracts can be stored at -20°C if samples shall be processed later.

Protocol 3: DNA extraction from complex liquid specimens

This protocol describes the workflow of extracting DNA from up to 500µL of complex liquid samples, such as blood, sputum (see also section Application Notes), tissue homogenates, transport media containing alcohols, and liquid-based cytology media. This protocol includes a cell capture step and a subsequent wash step before DNA extraction.

To do before starting

- ***Read the complete protocol.***
- Heat a water bath, heating block, or thermal shaker to 95°C.
- Shake or vortex Beads A for 30 seconds to ensure a homogeneous suspension.
- Prepare a sufficient amount of **"capture mix"** of Buffer BPE and Beads A for your extractions as follows:
    - Determine the volume of sample you want to process (200 – 500µL)
    - Pipette an equal volume of Buffer BPE into a tube, e.g. 500µL of Buffer BPE if you want to process 500µL of liquid sample)
    - Add 30µL of Beads A to the Buffer BPE
      Note: Do not modify the volume of Beads A! It is always 30µL - independent of the volume of Buffer BPE.
    - Mix well for 10 seconds by vortexing or inverting the tube.

1. **Add the prepared "capture mix" (see above) to a microcentrifuge tube.**

2. **Add the desired volume of liquid sample (200 – 500µL) to the "capture mix". Mix well by pipetting up and down or by vortexing or inverting the tube for 10 seconds.**

3. **Incubate the sample tube at room temperature for 3 minutes.**

   Note: If a maximum sensitivity is desired, the incubation time for cell binding can be extended up to 10 minutes.

4. **Mix well for 5 seconds, then place the sample tube into a magnetic stand and let the magnetic particles separate at room temperature for 2 minutes or until the beads are separated.**

5. **Open the lid while the tube remains in the magnetic stand. Remove the supernatant completely by pipetting. Discard the supernatant.**

6. **Add 500µL of Buffer BPE to the tube and remove the tube from the magnetic stand. Resuspend the magnetic particles by vortexing or shaking for 10 seconds.**

7. **Place the sample tube into a magnetic stand and let the magnetic particles separate at room temperature for 1 minute.**

8. **Open the lid while the tube remains in the magnetic stand. Remove the supernatant completely by pipetting. Discard the supernatant.**

9. **Add 100µL of Buffer BPL to the tube and remove the tube from the magnetic stand. Resuspend the magnetic particles by vortexing or shaking for 10 seconds.**

10. **Incubate the lysis mixture at 95°C for 10 minutes.**

    Note: If a thermal shaker is used, shake at maximum speed. The heating time can range from 5 to 15 minutes and should be validated by the user for the particular application.

11. **Remove the sample tube from the heating device and mix well for 5 seconds. Remove the condensate from the lid before opening by shaking down or tapping the tube on the work bench.**

12. **Open the lid and add 20µL Buffer AD to the lysis mixture. Mix well for 5 seconds by vortexing or inverting the tube.**

13. **Place the sample tube into a magnetic stand and let the magnetic particles separate at room temperature for 1 minute.**

14. **Open the lid while the tube remains in the magnetic stand and transfer the supernatant into a new tube for storage or use in downstream applications.**

DNA extracts can be stored at -20°C if samples shall be processed later.

Additional advice and application notes

Processing of viscous samples such as sputum

Very viscous samples are recommended to be liquefied before specimens should be liquefied by a product or method that is not based on sodium hydroxide or guanidinium salts. Suitable methods are DTT- or other sulfide-based products, e.g. SL Solution (Copan Diagnostics) or Sigma-SP (Medical Wire & Equipment).

Processing of increased volumes of liquid sample

You can increase the sample volume being processed with Protocols 2 and 3 beyond 1mL. For that, the volume of Buffer BPE applied must be increased as well to keep the ratio of sample and Buffer BPE as instructed in the respective Protocol. The amount of Beads A is always kept at 30µL. Additional Buffer BPE can be ordered separately.

Processing of dirty and complex samples

If a higher purity of the lysate is required, the bound cells can be washed multiple times. For that, repeat Steps 6 to 8 of Protocol 3. Additional Buffer BPE can be ordered separately.

It has been reported that an increase in time given for capturing of cells to up to 10 minutes can lead to higher sensitivity the DNA extraction procedure.

Increasing the time of magnetic separation to up to 5 minutes can lead to a slightly higher purity of the extracted DNA.

Capturing of intracellular parasites residing in red-blood cells

Red-blood cells (RBC) do not bind to Beads A and can therefore not be concentrated. If intracellular parasites in RBC, e.g. *Plasmodium falciparum*, are intended to be extracted, then the RBC need to be lysed before performing the extraction protocol by adding an appropriate RBC lysis buffer. The hemolyzed sample can then be subjected to DNA extraction using SwiftX DNA Protocol 3.

Limitations

Specimens stored in inactivating media

The cell capturing function of SwiftX™ ParaBact (see Protocols 2 and 3) cannot be utilized if the cells in the specimen have already been lysed. This can be the case if the sample has been transported or stored in an inactivating buffer with high concentration of chaotropic salt, because they tend to lyse biological cells and may reduce the number of intact cells available for capturing. Examples for such transport media are eNAT, PrimeStore MTM, and DNA/RNA Shield. In such case, you need to test whether the target cells are still intact, so they can be captured with Beads A.

Photometric determination of DNA concentrations, e.g. using NanoDrop

Although the reverse purification mechanism actively removes impurities and inhibitors, DNA extracted with SwiftX™ ParaBact is not as pure as nucleic acids extracted with silica-based purification kits. Thus, photometric determination of A260/A280 ratios and quantification by A260 measurement is of limited use. This is due to the lower limit of quantification of the Nanodrop method, which leads to wrong analysis data especially in samples with low cell count or DNA concentration. Measurements have a in general and may lead to misinterpretation of the extraction efficiency. If you still wish to perform a photometric analysis, do not forget to blank your measurement with Buffer DL instead of water or other buffers. Also, Buffer DL itself absorbs light around a wavelength of 280nm.

Analysis of extracted DNA using digital PCR

Buffer BPL contains low concentrations of detergents. It was observed that DNA samples extracted with SwiftX™ ParaBact should only be applied to digital PCR analysis if the dPCR system does not require droplet generation, because Buffer BPL interferes with droplet stability. If you still wish to use SwiftX™ ParaBact extracts for digital PCR, we recommend diluting the extracted DNA with water or another detergent-free buffer.

Contacts and disclaimer

This product is owned by:

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This product may be used solely in accordance with the provided protocol. Every step deviating from this protocol must be validated by the user.

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