SwabUp™ Lab Monitoring

For the regular monitoring of lab work area and detection of target and amplicon DNA contaminations

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Cat. No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

Small amounts of amplicon or target DNA contaminations represent a major issue in molecular biology, especially for highly sensitive techniques like PCR, and often lead to PCR artifacts and false positive results. Originating from aerosoles in centrifuges, pipettes, and other lab equipment or from accidental spills, contaminant DNA is very hard to remove and can lead to cross-contaminations between samples. A PCR amplification process can detect down to a single DNA molecule, imparing the testing procedure and causing misinterpretation of results and inaccurate data. Such contaminations can occur from time to time, even in experienced labs and will go unnoticed unless detected in PCR.

The SwabUp™ Lab Monitoring kit is designed to assist in the identification of DNA contamination hot-spots in molecular biology laboratories and as a tool for regular monitoring and cleaning of lab and work areas. These are, in fact, key strategies to ensure early detection, prevention, and efficient eradication of DNA contaminations.

The SwabUp™ Lab Monitoring kit combines the components needed for optimal sample collection and efficient DNA extraction to provide purified DNA, ready-to-use for PCR. In combination with a contamination-free PCR mix (see our ConviFlex™ DNAmp Mix, Cat. No. 191-0100) and user-provided primers, the SwabUp™ Lab Monitoring kit offers a competent system for environmental monitoring (e.g. centrifuges, pipettes, and any surface of a molecular lab work area, read more in chapter „Specimen“).

PRINCIPLE OF THE METHOD

The method is simple and consists of five general steps: (1) Collection of samples using the provided swab applicators, (2) selective binding of DNA to spin columns, (3) removal of residual contaminants and inhibitors, and (4) elution of purified DNA.

(1)

Collection swab applicators are packaged individually in sealed plastic peel-pouches. The shaft of the applicator is made of plastic and the top-end (tip) is made of flocked nylon fibers with excellent absorption ability. The shaft of the collection swab applicators has a molded breakpoint, which facilitates breakage after sample collection and transport into the tube containing the Collection Buffer. These swabs were specifically selected for this application after extensive testing and showed the best performance in this procedure, compared to several alternatives.

(2-4)

The DNA extraction procedure is necessary to eliminate potentially PCR-inhibiting components such as fabric or paper derivatives, dust, or high concentrations of proteins. However, the procedure is not based on phenol/chloroform extraction and requires minimal hands-on time (approx. 30 minutes), providing DNA ready-to-use for PCR. The DNA extraction system was optimized for efficient detection of the minimal amounts of contaminant DNA.

At this point, the collected samples can be analyzed by PCR or qPCR with any PCR system of choice. However, we strongly recommend a contamination-free PCR system to exclude potentially misleading results due to contaminated PCR reagents and buffers. Our ConviFlex™ DNAmp Mix (Cat. No. 191-0100) includes a lyophilized hot start Taq polymerase containing PCR mix, compatible with both conventional- and qPCR.

CONTENT

Each kit contains reagents and components for 10 or 50 samples. The expiry date of the unopened package is marked on the package label. Components of DNA extraction system must be stored at room temperature. Collection Buffer tubes must be stored at +2 – +8 °C immediately after delivery. Swabs can be stored at +2 – +30 °C.

Kit Component	10 Swab-Samples Cat. no. 181-0010	50 Swab-Samples Cat. no. 181-0050
Swabs	10 units	50 units
Collection Buffer tubes	10 units	50 units
Spin columns	10 units	50 units
Collection tubes	10 units	50 units
Starting Buffer	5 ml	15 ml
Binding Buffer	10 ml	25 ml
Buffer SW1	3 ml (add 3 ml ethanol, abs., before first use)	15 ml (add 15 ml ethanol, abs., before first use)
Buffer SW2	4 ml (add 16 ml ethanol, abs., before first use)	12 ml (add 48 ml ethanol, abs., before first use)
Elution Buffer	2 ml	2 × 2 ml

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com / www.minervabiolabs.us).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

SwabUp™ Lab Monitoring kit contains reagents and components for collection of samples and extraction of DNA. Additional consumables and equipment are supplied by the user:

- Ethanol > 96 % abs.
- DNase-free reaction tubes (1.5 ml or 2 ml)
- Microcentrifuge and heat block for 1.5 ml (or 2 ml) reaction tubes
- Pipettes with corresponding DNase-free filter tips (100 and 1000 µl)
- Components and equipment for PCR amplification (recommended: MB Taq DNA Polymerase or ConviFlex™ DNAmp Mix. See „Related Products“ for ordering information).

SPECIMEN

Different surfaces like desktops and lab work area, as well as equipment in molecular biological labs (e.g. centrifuge, pipettes, reaction tube racks etc.) are easily exposed to target and amplicon DNA contaminations. By touching doorknobs, paper, computer keyboard and -mouse before and after doing lab work, lab operators unintentionally carry over and spread DNA contaminations regardless of experience and precautions. In addition, applying certain PCR techniques such as two-step qPCR or nested conventional PCR increases the risk of causing amplicon DNA contaminations by carrying over DNA contaminants from one PCR to the next through pipetting.

Samples should therefore be collected from surfaces and/or equipment, which are more easily exposed to target and amplicon DNA contaminations, e.g., centrifuge, pipettes, reaction tube racks, doorknobs, lab books, computer keyboards and mouse, touchpad, desktops and any surface of a molecular lab work area.

Each sample should be collected by thorough swabbing with the swab top-end (tip) a distinct 10×10 cm surface area.

RECOMMENDATIONS

The SwabUp™ Lab Monitoring kit is recommended for the regular monitoring of the lab work areas and detection of target or amplicon DNA contaminations. Identification of DNA contamination hot-spots will help maintaining clean work areas and avoiding PCR artifacts or inaccurate data. To this aim, we recommend performing this test at regular time intervals. Also, we strongly recommend setting the PCR amplification with the most frequently used primer sets, or those most frequently associated with irregularities or unspecific results. This strategic measure helps verifying the presence of contaminations also in these reagents.

For your convenience, we have collected here (see Appendix I) specific instructions for lab monitoring, which are based on our long-term experience and extensive testing. A table for the documentation of the lab monitoring process, as well as initial guidelines for the necessary measures (in case of a DNA contamination) are included. These guidelines can be a great support in the process of tracking the contamination source and route and to take necessary measures for their elimination and prevention of reoccurrence.

SwabUp™ Lab Monitoring kit is for research use only. It is not recommended for clinical and diagnostic applications or for the detection of RNA contaminations.

PRECAUTIONS

The SwabUp™ Lab Monitoring kit should be used by trained laboratory staff only. All samples should be handled with all due care and attention. Always wear a suitable lab coat, goggles and disposable gloves.

The sample preparation waste contains Binding Buffer and Buffer SW1, which may form highly reactive compounds when combined with bleaching agents. DO NOT add bleaching agents or acidic solutions directly to the sample preparation waste. Clean with suitable laboratory detergent and water, if any liquid is spilled.

The Binding Buffer contains propan-2-ol and polyethylene glycol octylphenol ether and is, therefore, flammable, harmful and irritant. Buffer SW1 contains guanidinium thiocyanate and is, therefore, harmful and irritant. In case of skin or eye contact wash thoroughly with running water and seek medical attention immediately.

For more information, please read safety data sheets (SDS) on our website: www.minerva-biolabs.com.

ADDITIONAL NOTES

⇨ These instructions must be understood to successfully use the SwabUp™ Lab Monitoring kit. The reagents supplied should not be mixed with reagents from different lots but used as an integral unit. The reagents of the kit must not be used beyond shelf life.

⇨ To avoid DNA cross-contaminations during the process, the test should be performed under sterile and DNA-free conditions.

⇨ DNA extraction should be performed immediately after sample collection to avoid DNA cross-contaminations through storage.

⇨ Follow the exact protocol. Any deviation from the extraction method may affect the results.

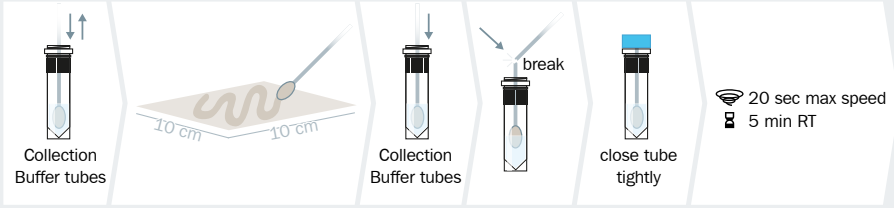
⇨ We recommend including control samples on a regular basis to monitor the reliability of your results. It is also advantageous in case of troubleshooting.

⇨ Do not use other alcohols apart from ethanol as it will lead to inconsistent yields.

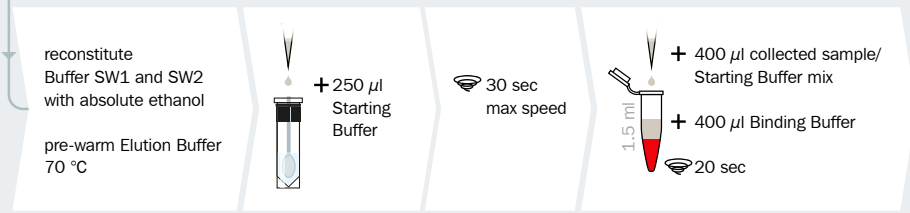
⇨ Pre-heating of Elution Buffer improves the DNA yield significantly.

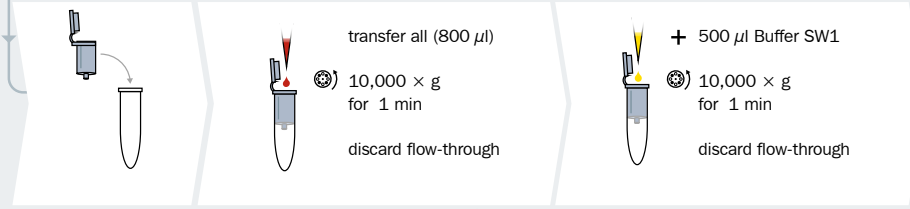
PROCEDURE - OVERVIEW

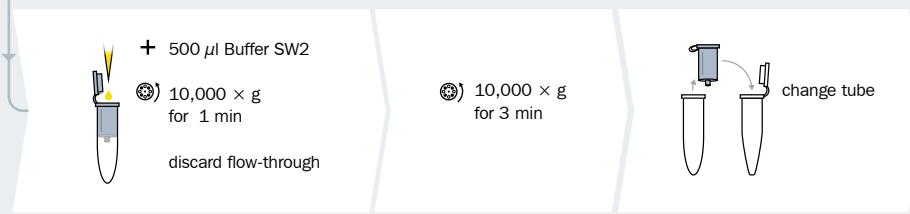
1. Sample Collection

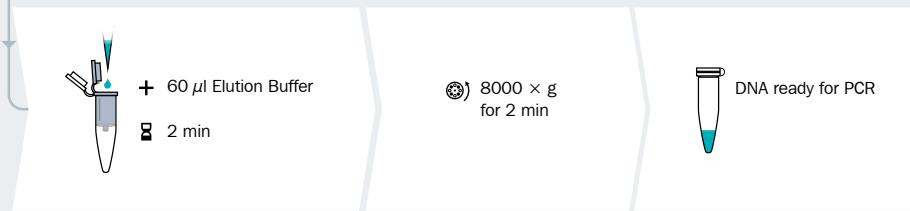


2. DNA Extraction









+ add vortex incubate centrifuge

This procedure overview is not a substitute for the detailed manual.

PROCEDURE - STEP BY STEP

1. Sample Collection

1.	Take out a swab applicator from the plastic peel-pouch by peeling the shaft-end of the pouch open. Note: you should always open the pouch at the shaft-end. Do not touch the tip of the swab during sampling.
2.	Open the Collection Buffer tube and dip the tip of the swab applicator into the **Collection Buffer** until it is completely soaked.
3.	Carefully take the soaked swab out of the tube and wipe the surface you wish to test thoroughly. A surface of 10 × 10 cm is recommended for optimal results.
4.	Transfer the swab applicator into the Collection Buffer tube. Use the molded breakpoint in the shaft of the swab applicator to break the shaft so that the top-end of the swab is left inside the tube.
5.	Close the tube tightly and vortex for 20 sec at maximum speed.
6.	Incubate samples at room temperature for 5 min. Samples are now ready for DNA extraction.

2. DNA Extraction

⇨ Before first use reconstitute Buffer SW1 and Buffer SW2 with absolute ethanol.
⇨ Set the heat block to 70 °C and equilibrate required amount of Elution Buffer to 70 °C.

1.	Add **250 µl of Starting Buffer** to the collected sample and vortex at maximum speed for at least 30 sec.
2.	Transfer 400 µl to a DNase-free 1.5 ml reaction tube and add **400 µl of Binding Buffer** to the sample. Vortex immediately and thoroughly in order to prevent any precipitation of nucleic acids. Do not centrifuge the sample and proceed immediately to step 3.
3.	Place a spin column in a collection tube. Transfer the Binding Buffer/sample - mix (approx. 800 µl) into the spin column. Note: be careful not to moisten the rim of the spin column.
4.	Centrifuge the spin column at ≥ 10,000 × g for 1 min. Discard the flow-through from the collection tube and reassemble spin column and collection tube.
5.	Add **500 µl of Buffer SW1**. Centrifuge the spin column at ≥ 10,000 × g for 1 min, discard the flow-through and reassemble the spin column and collection tube.
6.	Add **500 µl of Buffer SW2**. Centrifuge the spin column at ≥ 10,000 × g for 1 min, discard the flow-through and reassemble the spin column and collection tube.
7.	Centrifuge at full speed for 3 min in order to remove residual Buffer SW2.
8.	Discard the collection tube and place the spin column into a new DNase-free 1.5 ml reaction tube.
9.	Pipette 60 µl of pre-heated ***Elution Buffer*** (70 °C) into the spin column directly onto the center of the silica membrane. Be careful not to damage the membrane in the process. The membrane's surface should be completely covered with Elution Buffer.
10.	Incubate at room temperature for 2 min, then centrifuge at 8,000 × g for 2 min.
11.	The eluates can be used directly for PCR. If not analyzed immediately, eluates can be stored at +2 to +8 °C for a week or at ≤ -18 °C for long-term storage.

APPENDIX I

1. Lab monitoring and tracking of contamination hot-spots

In order to properly evaluate the efficiency of cleaning procedures in molecular biology laboratories, a comprehensive lab- and environment monitoring program should be carried out at 3-month intervals. A table to keep track of the monitoring process is shown below.

Date	Lab operator/s	Lab	Room/s No.

Sample No.	Sample-label	Collection spot/area	Ct-value 1 (sample)	Ct-value 2 (internal control**)	Band (conventi-onal PCR)	Result
1						
2						
3						
4						
5						
6						
7						
8						
9						
10	DNA extraction control*					
DNA amplification controls	Positive control					
	Negative control					
Evaluation and selected measures						

* The DNA extraction control is optional but we strongly recommend including it in the testing to verify the success of the extraction procedure.

** The internal control is optional and can be used for the validation of PCR amplification.

2. Measures for elimination and prevention of DNA contaminations

If a DNA contamination is detected in your lab, please proceed as follows:

1. If the contaminated sampled area is normally subjected to the regular lab cleaning procedure, the cleaning procedure should immediately be repeated using a sodium hypochlorite-containing cleaner for surfaces (or an adequate alternative for sensitive surfaces).

2. Measures to improve the cleaning procedure should be reviewed and put in place.

3. If the contaminated sampled area is NOT part of the regular lab cleaning procedure, this should be immediately reconsidered and this area included in the lab cleaning routine. Make sure to inform all lab operators and provide adequate training.

4. After cleaning, the PCR amplification should be repeated until contaminations cannot be detected anymore.

5. Make sure all lab operators are well aware of these measures and trained accordingly.

The lab monitoring template (see table on previous page) is also available for download on the website of this product.
Visit www.minerva-biolabs.com for more information and resources.

APPENDIX II

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising from the use, the results of use, or the inability to use this product.

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Related Products

PCR Mix		
191-0025/-0100/-0250	ConviFlex™ DNAmp Mix, PCR Mix with Taq polymerase for conventional and qPCR	25/100/250 reactions
192-0025/-0100/-0250	ConviFlex™ RT-Taq Mix, RT-PCR Mix with Taq polymerase and retrotrascriptase for conventional and RT-qPCR	25/100/250 reactions
PCR Clean™		
15-2025/-2200	DNA Decontamination Reagent, Spray bottle/refill bottles	250 ml/4 × 500 ml
15-2001	DNA Decontamination Reagent, Wipes in a dispenser box	50 wipes
15-2002	DNA Decontamination Reagent, Wipes in refill bags	5 × 50 wipes
LabClean™		
15-4100	DNA Decontamination Reagent, bottle	1 l
PCR Cycler Validation/Qualification		
57-2201	qPCR Cycler Check™	100 reactions
57-2102	PCR Cycler Check™ Advance	100 reactions
57-2103	PCR Cycler Check™ OneStep	100 reactions
MB Taq DNA Polymerase		
53-0050/-0100/-0200/-0250	MB Taq DNA Polymerase (5 U/µl)	50/100/200/250 units
53-1050/-1100/-1200/-1250	MB Taq DNA Polymerase (1 U/µl)	50/100/200/250 units
Nucleic Acid Extraction		
601-1010/-1050	ExtractNow™ DNA Mini Kit	10/50 extractions
602-1010/-1050	ExtractNow™ Blood DNA Mini Kit	10/50 extractions
603-1010/-1050	ExtractNow™ RNA Mini Kit	10/50 extractions
604-1010/-1050	ExtractNow™ CleanUp Kit	10/50 extractions
605-1010/-1050	ExtractNow™ Plasmid Mini Kit	10/50 extractions
606-1010/-1050	ExtractNow™ Virus DNA/RNA Kit	10/50 extractions
611-2250	ExtractNow™ Virus RNA Swab Kit	250 extractions
WaterShield™		
15-3015/-3020/-3050	Water Disinfection Additive for incubators and water baths, 200 × concentrate	15 × 10 ml/3 × 50 ml/500 ml
ZellShield™		
13-0050/-0150	Contamination Prevention Reagent 100× concentrate	50 ml/5 × 50 ml
Mycoplasma Elimination		
10-0200/-0500/-1000	Mynox® Mycoplasma Elimination Reagent	2/5/10 treatments
10-0201/-0501/-1001	Mynox® Gold Mycoplasma Elimination Reagent	2/5/10 treatments
Contamination Control Kits for conventional PCR		
11-1025/-1050/-1100/-1250	Venor®GeM Classic Mycoplasma Detection Kit	25/50/100/250 reactions
11-7024/-7048/-7096/-7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 reactions
11-8025/-8050/-8100/-8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 reactions
12-1025/-1050/-1100/-1250	Onar® Bacteria Detection Kit	25/50/100/250 reactions
Contamination Control Kits for qPCR		
11-9025/-9100/-9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 reactions
11-91025/-91100/-91250	Venor®GeM qOneStep Mycoplasma Detection Kit	25/100/250 reactions

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