

SwabUp™ Lab Monitoring Plus

DNA sample collection kit with a contamination-free PCR system for the regular monitoring of lab work area

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols



Lot No.



Order No.



Expiry date



Storage temperature



Number of reactions



Manufacturer

INDICATION

Small amounts of amplicon- or target DNA contaminations could lead to PCR artifacts and false positive results in the highly sensitive PCR technique. Originating from aerosolized fragments in centrifuges, pipettes and other lab equipment or from small splatters during working with open reaction tubes, contaminant DNA is very hard to remove and can lead to cross contaminations between samples. A single DNA molecule can be detected in the amplification process leading to widespread problems throughout the testing procedure and interpretation of results. Unfortunately, DNA contaminations can occur occasionally even in experienced labs and will go unnoticed unless detected in PCR.

The purpose of SwabUp™ Lab Monitoring kits is the tracing of DNA contamination hot-spots in molecular biology labs in order to efficiently eliminate them and prevent future occurrence. Regular cleaning and monitoring of lab and work area can help the early detection and avoidance of DNA contaminations. SwabUp™ Lab Monitoring kits combine components for sample collection, DNA extraction and PCR amplification and therefore exhibit a competent system for environment-monitoring.

SwabUp™ Lab Monitoring Plus kit is very easy to use. 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, exhibiting excellent absorption ability. The collection swab applicators have a molded breakpoint in the shaft of the applicator, which facilitates easy breakage of the swab applicator after collecting the sample and transport into the tube containing the Collection Buffer. After extensive testing, these swabs were specifically selected for the purpose of this kit, as they have proven to be especially suitable for the procedure. The DNA extraction system was optimized for the efficient detection of the smallest amounts of contaminant DNA. In addition, a contamination-free ready-to-use PCR system is added, SwabUp™ DNAmix, to exclude DNA cross-contaminations which are caused by user's own potentially contaminated PCR reagents and buffers.

PRINCIPLE OF THE METHOD

The method is simple and consists of the following 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, (4) elution of purified DNA, and (5) PCR amplification using the lyophilized SwabUp™ DNAmix.

The DNA extraction procedure is necessary in order to avoid PCR inhibition through inhibiting substances such as fabrics, tissues, dust or a high protein content of the collected sample. It should therefore be performed prior to the analysis of collected samples through PCR amplification. The procedure does not require phenol/chloroform extraction, and both DNA extraction and PCR preparation need minimal hands-on time. DNA extraction is completed in ~30 minutes, and setting the PCR reaction requires only a few minutes due to the ready-to-use PCR mix.

Lyophilized SwabUp™ DNAmix was designed as a universal PCR mix, compatible with both conventional and qPCR (both SYBR Green and TaqMan based techniques). Due to its broad range applicability, it can easily be adapted to most PCR protocols, programs and cycler types.

REAGENTS AND COMPONENTS

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. SwabUp™ DNAmix should be stored at 2 – 8 °C, and after rehydration at ≤ -18 °C. PCR mix is stable even after frequent freeze/thaw cycles.

Kit Component	10 Swab-Samples (182-0010)	50 Swab-Samples (182-0050)
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
SwabUp™ DNAmix (contains hot start Taq polymerase and dNTPs)	1 vial, freeze-dried (for 25 reactions)	5 vials, freeze-dried (for 25 reactions each)
2× Rehydration Buffer	1 vial, 1 ml	2 vials, 1 ml each
MgCl ₂ (100 mM)	1 vial, 1 ml	1 vial, 1 ml

The lot-specific QC certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The SwabUp™ Lab Monitoring Plus kit contains reagents and components for collection of samples, DNA extraction, and PCR amplification. 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 (10, 100 and 1000 µl)

- PCR reaction tubes and caps
- PCR or qPCR thermocycler (depending on desired amplification and detection method)

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 how experienced and careful they are. 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 easily exposed to target and amplicon DNA contaminations, e.g., centrifuge, pipettes, reaction tube racks, door-knobs, lab books, computer keyboard, computer mouse, touchpad, desktops and any surface of a molecular lab work area.

Each sample should be collected by using the swab top-end and thoroughly swabbing a different 10 × 10 cm surface.

RECOMMENDATIONS

SwabUp™ Lab Monitoring kit is recommended for the regular monitoring of the lab work area and detection of target or amplicon DNA contaminations. Detection of DNA contamination hot-spots will help maintain a clean work area and avoid PCR artifacts and inaccurate data. Therefore, we recommend performing this test in regular time intervals. It is also recommended to set the PCR amplification with the most frequently used primer sets, or those with the most frequent reoccurrence of irregularities or unspecific results.

For your assistance we provide you with instructions for lab monitoring, which were set up after years of experience and extensive testing, a table for the documentation of the lab-monitoring process, as well as initial guidelines for the measures which should be taken in case of a DNA contamination (s. Appendix I). By following these instructions and guidelines you will be able to track the source of DNA contaminations in your lab and the route on which they are carried over, and take necessary measures to eliminate these contaminations and prevent future occurrence.

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 Plus 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 spilt.

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.

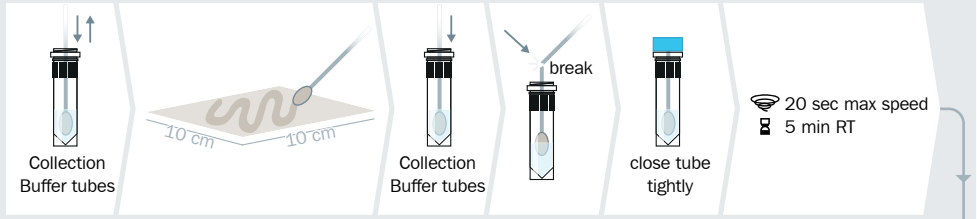
For more information please read safety data sheets (SDS) on our website: www.minerva-biolabs.com

ADDITIONAL NOTES

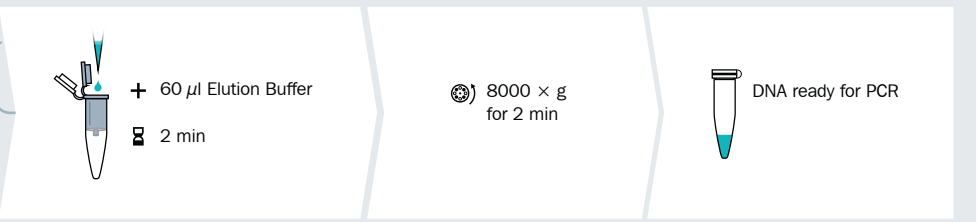
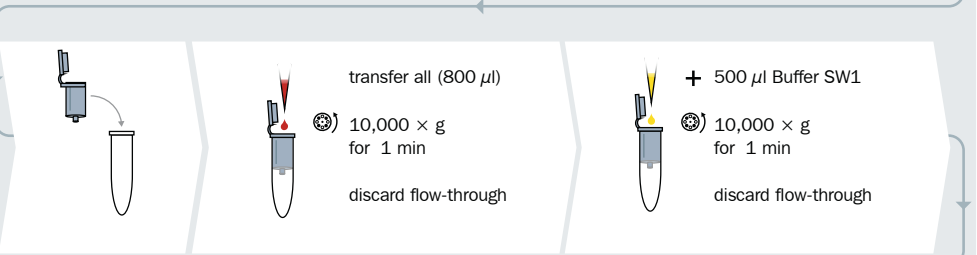
-
- ⇒ These instructions must be understood to successfully use the SwabUp™ Lab Monitoring Plus kit. The reagents supplied should not be mixed with reagents from different lots but used as an integral unit. The reagents of the kit must not be used beyond shelf life.
-
- ⇒ To avoid DNA cross-contaminations during the process, the test should be performed under sterile and DNA-free conditions.
-
- ⇒ DNA extraction and PCR amplification should be performed immediately after sample collection to avoid DNA cross-contaminations through storage.
-
- ⇒ Follow the exact DNA extraction protocol.
-
- ⇒ Do not use other alcohols apart from ethanol as it will lead to inconsistent yields.
-
- ⇒ Pre-heating of Elution Buffer improves the yield significantly.
-
- ⇒ We recommend including control samples on a regular basis to monitor the reliability of your results. It is also advantageous in case of troubleshooting.
-
- ⇒ Set up at least one negative control sample (non-template control, NTC) in each PCR. Use the elution buffer from the DNA extraction system to set up the NTC.
-
- ⇒ The control samples must be processed in the same manner as the test samples. You may want to include other laboratory specific control samples such as high, median and low DNA levels.
-

PROCEDURE - OVERVIEW

1. Sample Collection



2. DNA Extraction



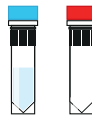
+ add vortex incubate centrifuge

3. Preparation of PCR Mastermix

⊕ SwabUp™ DNAmP Mix
5 sec max speed

Rehydration Buffer

260 μ l



SwabUp™ DNAmP Mix

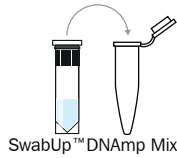
⌚ 5 min RT

⊕ briefly

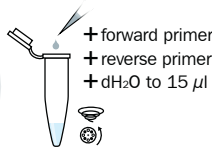
⊕ 5 sec

4a. Preparation of PCR Reactions (Conventional PCR)

transfer required amount

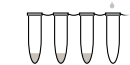


SwabUp™ DNAmP Mix



+ forward primer
+ reverse primer
+ dH₂O to 15 μ l

loading
reaction tubes



aliquot 15 μ l

+ 5 μ l DNA Extract
+ 5 μ l Positive Control
+ 5 μ l PCR grade water (NC)

⊕ briefly
⊕ 5 sec

5a. PCR Amplification

Example

1 cycle 94 °C for 2 min
25 cycles 94 °C for 30 sec
55-68 °C for 15-30 sec*
72 °C for 60 sec / 1 kb target
hold 4 - 10 °C

*Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers.

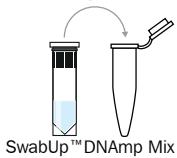
Gel electrophoresis - example



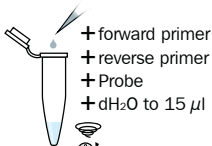
DNA Ladder
Positive Control
Negative Control
Positive sample 1
Positive sample 2
Negative sample

4b. Preparation of PCR Reactions (qPCR)

transfer required amount

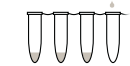


SwabUp™ DNAmP Mix



+ forward primer
+ reverse primer
+ Probe
+ dH₂O to 15 μ l

loading
reaction tubes



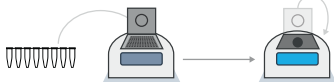
aliquot 15 μ l

+ 5 μ l DNA Extract
+ 5 μ l Positive Control
+ 5 μ l PCR grade water (NTC)

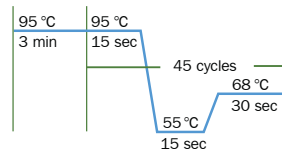
⊕ briefly
⊕ 5 sec

5b. qPCR Amplification

Example



Start PCR program



+ add ⌚ incubate ⊕ centrifuge ⊕ vortex

NC = Negative Control

NTC = Non-Template Control

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

MB_SI_SwabUp-Kit-Plus_02_EN

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. Take the soaked swab out of the tube carefully 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 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 with 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 $\geq 10,000 \times g$ 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 $\geq 10,000 \times g$ 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 $\geq 10,000 \times g$ 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.
-

-
- 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 covered with Elution Buffer.
-
9. Incubate at room temperature for 2 min, then centrifuge at 8,000 \times g for 2 min.
-
10. 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 \leq -18 °C for long-term storage.
-
- 11.
-

3. DNA Amplification

- ⇒ All samples including positive and negative controls should be set up in duplicates.
- ⇒ All reagents should be equilibrated to 2 - 8 °C. After reconstitution, SwabUp™ DNAmix should be stored at \leq -18 °C.

3.1. Preparation of PCR mix

-
1. Spin down lyophilized **SwabUp™ DNAmix** (red cap) for 5 sec at max. speed.
-
2. Add **260 μl 2 \times Rehydration Buffer** (blue cap).
-
3. Incubate PCR mix at room temperature for 5 min.
-
4. Vortex PCR mix briefly and spin down for 5 sec.
-

Proceed to 3.2. for conventional PCR or to 3.3. for qPCR amplification.

- ⇒ Attention: The following steps are only examples for PCR and qPCR protocols. You should always adjust volumes and concentrations to the requirements of your assay. Please note, that this PCR system was designed and tested for 20 μl reaction setup. You should therefore prepare your PCR mastermix accordingly and add all necessary components to a final-volume of 15 μl per reaction. We recommend a sample volume of 5 μl DNA-extract / reaction. Final concentration of MgCl_2 is 2.4 mM in 20 μl reaction (recommended MgCl_2 concentrations are 1.5 mM - 6 mM).

3.2. Preparation of PCR reactions (conventional PCR)

-
1. Transfer the required amount of reconstituted **SwabUp™ DNAmix** (according to number of samples you are testing for one set of primers) into a new DNase-free 1.5 ml tube.
-
2. Add 0.1 - 0.5 μM forward primer and 0.1 - 0.5 μM reverse primer.
Optional: add MgCl_2 , if necessary, to optimize your assay.
-
3. Add PCR grade water to a final volume of 15 μl per reaction, and mix by tapping carefully against the tube or pipetting up and down 4 - 5 times.
-
4. Aliquot 15 μl PCR mastermix to each PCR reaction tube.
-
5. Negative Controls: add 5 μl PCR grade water or elution buffer.
-
6. Samples: add 5 μl DNA extract.
-
7. Positive Control: add 5 μl template DNA which is specifically amplified with the primer set used.
-

8. Close PCR tubes tightly and spin down. Proceed to 3.4.

3.3. Preparation of PCR reactions (qPCR)

1. Transfer the required amount of reconstituted **SwabUp™ DNAmP Mix** (according to number of samples you are testing for one set of primers) into a new DNase-free 1.5 ml tube.
 2. Add 0.1 - 0.5 μ M forward primer and 0.1 - 0.5 μ M reverse primer. Add 0.1 - 0.5 μ M probe, if you are using the TaqMan based qPCR technique.
 3. Optional: add MgCl₂, if necessary, to optimize your assay.
 4. Add PCR grade water to a final volume of 15 μ l per reaction, and mix by tapping carefully against the tube or pipetting up and down 4 - 5 times.
 5. Aliquot 15 μ l PCR mastermix to each PCR reaction tube.
 6. Non-Template Controls: add 5 μ l PCR grade water or elution buffer.
 7. Samples: add 5 μ l DNA extract.
 8. Positive Control: add 5 μ l template DNA which is specifically amplified with the primer set used.
 9. Close PCR tubes tightly and spin down. Proceed to 3.4.
-

3.4. Start PCR amplification

1. Place PCR tubes in the cycler and close the lid tightly.
 2. Program the PCR or qPCR cycler or check stored temperature profiles (see Appendix II for detailed PCR protocols and cycler programs of selected qPCR cyclers).
 3. Start the program.
-

DATA INTERPRETATION

- ⇒ Detection of the positive control is an indication for the success of PCR amplification.
- ⇒ Detection of the negative control indicates a cross contamination occurred during PCR process. PCR amplification should be repeated.

Detection of Positive Control	Detection of Negative Control	Detection of Sample	Interpretation
positive	positive	irrelevant	repeat PCR
negative	irrelevant	irrelevant	repeat PCR
positive	negative	positive	DNA contamination
positive	negative	negative	No DNA contamination

PRODUCT CHARACTERISTICS (SwabUp™ DNAmix)

1. Hot start Taq polymerase

SwabUp™ DNAmix contains a hot start Taq polymerase which is suitable for a wide range of applications, highly sensitive (detection of ≥ 6 DNA molecules), and can effectively amplify DNA templates up to 5 kb in length at an extension rate of 1 kb / 1 min.

2. Applicability

SwabUp™ DNAmix was tested using several different assays and showed a broad range compatibility and excellent characteristics with regard to applicability and convenience. SwabUp™ DNAmix showed functional compatibility when tested in PCR amplification of bacterial and human genomic DNA, in conventional and qPCR, in both TaqMan- and SYBR Green based techniques, and on two different qPCR cyclers from different manufacturers, RotorGene® 6000 (Corbett) and CFX96 Touch™ (Bio-Rad), applying both two-step and three-step PCR programs (s. Appendix II for details and examples).

3. Stress- and storage-testing

Temperature stability of the lyophilized SwabUp™ DNAmix was tested at room temperature, 4 °C, 37 °C, and 60 °C for 1, 3, or 7 days. Functionality of PCR mix was positive for all temperatures and time points tested. No sensitivity loss was observed for any of the tested temperatures.

4. In-use stability testing

Functionality of rehydrated SwabUp™ DNAmix was tested after several freeze/thaw cycles and compared with that of a fresh mix. PCR mix was stable even after 5 freeze/thaw cycles, whereat no sensitivity loss was observed and fluorescence intensity was constant.

A detailed Technical Note is available upon request. Please find contact information on our homepage: www.minerva-biolabs.com

APPENDIX I

1. Lab Monitoring and tracking of contamination hot-spots

In order to supervise the efficiency of cleaning procedures in molecular biology labs, a comprehensive lab- and environment monitoring should be carried out in 3-month intervals.

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 (conventional PCR)	Result
1						
2						
3						
4						
5						
6						
7						
8						
9						
10	DNA extraction control*					
DNA amplification controls	Positive control					
	Negative control					

Evaluation and measures						

* DNA extraction control is optional but we recommend including it in the testing for the verification of the extraction procedure.

** 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 was detected in your lab, you should proceed as follows:

1. If a DNA contamination was detected within an area, which is part of the regular cleaning procedure of your lab, you should immediately repeat the procedure using a sodium hypochlorite-containing surface cleaner (you should seek an adequate alternative for sensitive surfaces).
2. You should revise and take measures to improve the cleaning procedure in place.
3. If a DNA contamination was detected within an area, which is not part of the regular cleaning procedure of your lab, you should immediately include this area in your lab cleaning routine. Inform all lab operators and perform a proper training.
4. Repeat PCR amplification after cleaning until no contamination can be detected anymore.
5. Make sure all operators at your lab are well aware of these measures, and trained accordingly.

The above-provided table is available for download on our homepage: www.minerva-biolabs.com

APPENDIX II

The following PCR programs are examples based on our own experience, which are meant to assist you with the use of the PCR system. You should always adjust temperatures and incubation time to the requirements of your own assay.

Program for conventional PCR cycler

Thermocyclers should be programmed according to manufacturer's instructions.

Step	Temperature	Time	Cycles
Initial denaturation	94 °C	2 min	1
Denaturation	94 °C	10 sec	25 - 35
Annealing	55 - 68 °C *	15 - 30 sec	
Extension	72 °C	60 sec / 1 kb of target	
Final extension	72 °C	5 - 10 min	1
Hold	4 °C	indefinitely	1

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers.

Programming the RotorGene® 6000

The following two-Step- and three-step-PCR programs are examples for PCR amplification of human genomic DNA using SwabUp™ DNAmix. This PCR amplification was successfully tested on RotorGene® 6000 cycler using both TaqMan- and SYBR Green-based techniques.

1. Two-Step PCR Program

Program Step 1: Pre-incubation

Setting	Hold
Hold Temperature	95 °C
Hold Time	3 min 0 sec

Please check the correct settings for the filter combination:

green filter (510):	target DNA
yellow filter (555):	Internal Control

Program Step 2: Amplification

Setting	Cycling
Cycles	45
Denaturation	95 °C for 15 sec
Annealing	55 °C for 30 sec → acquiring to Cycling A (green and yellow)
Gain setting	automatic (auto Gain)
Slope Correct	activated
Ignore First	deactivated

2. Three-Step PCR Program

Program Step 1: Pre-incubation

Setting	Hold
Hold Temperature	95 °C
Hold Time	3 min 0 sec

Please check the correct settings for the filter combination:

green filter (510):	target DNA
yellow filter (555):	Internal Control

Program Step 2: Amplification

Setting	Cycling
Cycles	45
Denaturation	95 °C for 15 sec
Annealing	55 °C for 15 sec → acquiring to Cycling A (green and yellow)
Elongation	68 °C for 30 sec
Gain setting	automatic (auto Gain)
Slope Correct	activated
Ignore First	deactivated

Result Reading:

- Open the menu *Analysis*
- Select *Quantitation*
- Check the required filter set (green and yellow) according to the following table and start data analysis by double click.

- The following windows will appear:
Quantitation Analysis - Cycling A (green or yellow)
Quant. Results - Cycling A (green or yellow)
Standard Curve - Cycling A (green or yellow)
- In window *Quantitation Analysis*, select first *linear scale* and then *slope correct*
- Threshold setup (not applicable if a standard curve was included in parallel and auto threshold was selected)
 - In window *Ct Calculation* set the threshold value to 0-1
 - Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- The Ct-values can be taken from the window *Quant. Results*.

Programming the CFX96 Touch™, CFX96 Touch Deep Well™, CFX Connect™, and CFX384 Touch™ (Bio-Rad)

The following two-Step- and three-step-PCR programs are examples for PCR amplification of bacterial genomic DNA using SwabUp™ DNAmix. This PCR amplification was successfully tested on CFX96 Touch™ cycler using the TaqMan-based technique.

Performing Runs

Run Setup - Protocol Tab

- Click **Create New** to open the Protocol Editor to create a new protocol.
- Select any step in either the graphical or text display. The selected step becomes highlighted in blue. Click the temperature or incubation time to directly edit the value.

1. Two-Step PCR Program

	Step 1	Step 2	Step 3	Step 4
Temperature	95.0 °C	95.0 °C	55 °C	40 - 45 cycles
Incubation time	03:00 min	00:15 sec	00:30 sec	

2. Three-Step PCR Program

	Step 1	Step 2	Step 3	Step4	Step 5
Temperature	95.0 °C	95.0 °C	55.0 °C	68.0 °C	40 - 45 cycles
Incubation time	03:00 min	00:15 sec	00:15 sec	00:30 sec	

Run Setup - Plate Tab

- Click **Create New** to open the Plate Editor to create a new plate.
- Use the **Scan Mode** dropdown menu in the Plate Editor toolbar to designate the data acquisition

on mode to be used during the run. **Important!!!** Select the **All Channels** mode.

- Click the **Select Fluorophores** button to indicate the fluorophores that will be used in the run.
- Select the wells to be loaded within the plate diagram.

Run Setup – Start Run Tab

- View the selected Protocol file, Plate file, and data acquisition Scan Mode setting in the **Run Information** pane.
- Select one or more blocks and edit run parameters if necessary in Start Run on Selected Block(s) pane.
- Click the Start Run button to begin the run.

Data Analysis

Quantification Tab

The amplification chart data in this tab display the relative fluorescence (RFU) collected from each well at every cycle of the run.

- Choose the fluorophore data you want to display by clicking the fluorophore checkboxes located under the amplification chart.

Data Analysis Settings

- The Software uses two modes for quantification cycle determination. Select **Settings** from the menu bar and select **Baseline Subtracted Curve Fit** as baseline setting and **Single Threshold Mode** as Cq Determination Mode.
- In the **Single Threshold Mode**, click and drag the threshold line to manually position the line. Adapt the threshold line to the initial linear section of the positive control reaction.
- Samples showing no Ct-value can be considered as negative.

APPENDIX III

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

ConviFlex™ DNAmix

191-025/100/250 PCR Mix with Taq polymerase for conventional and qPCR 25/100/250 reactions

SwabUp™ Lab Monitoring Kit

181-0010/0050 Sample collection and DNA extraction 10/50 samples

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

Lab Clean™

15-4100 Molecular microbiology lab cleaner, bottled 1 Liter

PCR Clean™

15-2025/2200 DNA Decontamination Reagent, spray bottle/refill bottles 250 ml/4x 500 ml

15-2201 Wipes 120 wipes in a dispenser box

15-2202 Wipes, refill packs 5 x 120 wipes in a bag

15-2203 Wipes, single wrapped 30 sachets

WaterShield™

15-3015/3020/3050 Water Disinfection Additive for incubators and water baths, 200x concentrate 30 x 5 ml/3 x 50 ml/500 ml

PCR Cycycler Validation

57-2102 PCR Cycycler Check™ Advance 6 strips, 8 vials each

57-2103 PCR Cycycler Check™ OneStep 100 reactions

57-2202 qPCR Cycycler Check™ 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

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