EchoLUTION™ Buccal Swab DNA Kit

Spin column kits

USER MANUAL

RUO

REF

010-010-050

010-010-250

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1. INTENDED PURPOSE

The BioEcho EchoLUTION Buccal Swab DNA Kit is intended for easy, rapid, and efficient genomic DNA (gDNA) extraction from buccal swab samples. The excellent yield and purity of buccal cell DNA obtained with the EchoLUTION Buccal Swab DNA Kit allows use in downstream applications without further processing.

The EchoLUTION Buccal Swab DNA Kit is intended for research use only.

2. EXPLANATION OF THE KIT

The EchoLUTION Buccal Swab DNA Kit is characterized by the EchoLUTION single-step purification technology and an ultra-fast lysis step. Together, these reduce the lysis step to 2 minutes, reducing the overall extraction time and result in consistent quality compared to state-of-the-art methods.

The EchoLUTION Buccal Swab DNA Kit benefits are:

- Short processing time
- Ultra-fast lysis
- Few protocol steps
- High sample throughput with minor equipment and capital investment
- Up to 70 % less plastic waste compared to conventional methods
- Less toxic reagents

For further details about kit specifications, see Table 1.

Table 1: ***Kit specifications***

Specification	Description
Sample input	Buccal cells
Sample type	Dry swabs
Sample condition	Fresh or stored
Purified nucleic acid	Genomic DNA
Elution volume	100 µL
Expected yields	1–3 µg

2.1. *Single-step purification principle*

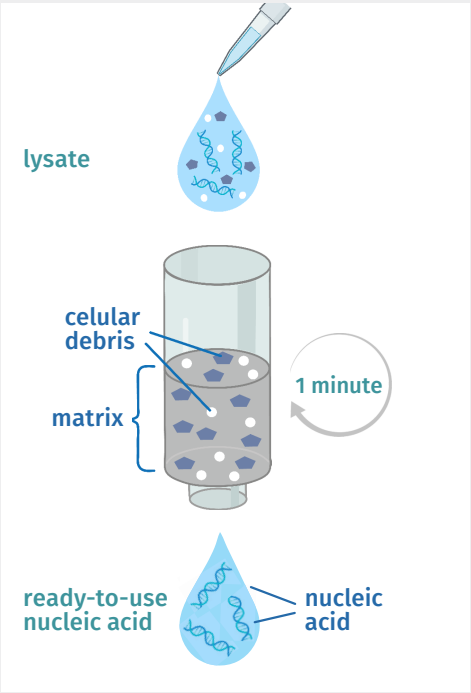
All BioEcho nucleic acid extraction kits are based on the EchoLUTION technology, which consists of tailored sample processing that includes lysis followed by single-step purification. The purification step works differently than conventional methods such as magnetic-bead and silica kits based on the bind–wash–elute method, and therefore needs only one centrifugation step.

The lysate is transferred onto the spin column or plate.

In a one-minute centrifugation step, nucleic acids pass through the purification matrix without interaction.

Impurities are held back and thereby completely removed.

The nucleic acids are in the flow-through and ready-to-use.



2.2. *General comments*

Comparison of the EchoLUTION™ technology to silica methods—general aspects and handling

Using the EchoLUTION technology, nucleic acids are not bound to a membrane or magnetic beads and can migrate freely through the filter matrix. Unwanted components of the lysate are removed from the sample by remaining in the purification matrix.
The advantages of the EchoLUTION technology are:

1. No time-consuming wash steps
2. Easy handling
3. Reduced plastic waste

In contrast, silica technologies are based on the principle of concentration. Here, the nucleic acids present in the lysate bind to a silica surface (membrane, magnetic beads), while unwanted cell components are removed by repeated washing with chaotropic and alcohol-containing wash buffers. Eventually, the nucleic acids are eluted with an aqueous buffer in the desired volume. Due to the repeated washing steps, silica-based methods are time-consuming, labor-intensive and environmentally unfriendly.

Handling of the purification matrix

The EchoLUTION purification matrix within the Spin Columns must be kept undamaged to avoid short-circuit currents. Short-circuit currents result in the introduction of lysis components into the eluate and inadequate purification, which can lead to inhibition in downstream analysis. Therefore, when applying the lysate to the column, **avoid touching the surface** of the filter matrix, and pipet **the sample very slowly (ideally dropwise)**.

To guarantee proper handling of the Spin Columns, be sure to use the recommended *g*-force centrifuge settings. Most centrifuges offer the choice between rpm and *g*-force (rcf); if not, calculate the rpm corresponding to the required *g*-force using the calculator in the link or the QR code below:

http://www.geneinfinity.org/sp/sp_rotor.html

For support on suitable centrifuges, please contact us.

Handling DNA

In general, cautious sterile microbiological practices should always be used when working with DNA. The most common sources of contamination are dust and hands, as they can hold bacteria and molds. Therefore, pay attention to what you're doing and always wear gloves while handling reagents and samples. Replace gloves regularly and keep tubes closed when possible.

Bench surfaces, laboratory equipment (e.g., pipettes and electrophoresis tanks) and non-disposable plasticware can be decontaminated using general laboratory reagents. Plasticware can be decontaminated with commercially available DNase removing solutions.

It is recommended to store purified DNA in a tightly-capped tube at 2–8 °C for 24 hours or at –20 °C for long-term storage.

3. MATERIALS

3.1. Materials provided

The kit contains a lysis buffer and a lysis solution that require preparation before they can be used for the first time. Please read section 4.1 before starting your DNA extraction.

Table 2: ***Content of EchoLUTION Buccal Swab DNA Kit; spin column format***

Product number	010-010-50	010-010-250
Product name	EchoLUTION Buccal Swab DNA Kit (50)	EchoLUTION Buccal Swab DNA Kit (250)
Reactions	50	250
Lysis Buffer Buccal Swab* (LB)	1 × 280 mg	2 × 700 mg
Lysis Solution Buccal Swab* (LS)	1 × 20 mL	2 × 50 mL
Clearing Solution Buccal Swab (CS)	2 × 0.75 mL	1 × 7.5 mL
Low-TE Buffer (T)	1 × 1.2 mL	2 × 1.2 mL
Spin Columns Buccal Swab	50	250

**For correct preparation of these components, read section 4.1*

3.2. Materials required but not provided

A. Microcentrifuge tubes

Use a 2 mL tube for Spin Column preparation and a 1.5 mL tube for sample lysis and elution.

3.3. Optional materials

A. BioEcho Cap Puncher

Alternatively, the cap puncher can be used for convenient handling of Spin Columns. To purchase this item, use the product number 050-001-001.

B. Spin Column Adapter for Plate Centrifuges

If you want to use a plate centrifuge for spin columns and avoid the standing time of the Spin Columns mentioned in the protocol, we suggest using a swing-out rotor centrifuge with our Spin Column Adapter for Plate Centrifuges (product number: 050-011-024).

3.4. Laboratory equipment needed

A. Microcentrifuge

Centrifugation can be performed in a microcentrifuge with a rotor for 2 mL reaction tubes. The centrifuge must be capable of reaching 1,000 x *g*. When using a plate centrifuge, please use the Spin Column Adapter for Plate Centrifuges offered by us (product number: 050-011-024).

B. Pipetting equipment

Pipetting can be performed using a single-channel pipette. We recommend using wide-bore tips for mixing and transferring the lysate to the purification matrix.

C. Thermal shaker

The thermal shaker used for the lysis step must be capable of reaching 80 °C and 1,400 rpm (e.g., Eppendorf® ThermoMixer® C). Alternatively, you can use a heating block or heat chamber.

D. Vortex mixer

A vortex mixer is required for lysate mixture.

E. Side cutter or scissors

The side cutter or scissors are necessary to cut the swab heads off. Please, remember to clean and disinfect your cutting tool before using it.

4. STORAGE AND STABILITY

4.1. Kit reagents

- The EchoLUTION Buccal Swab DNA Kit is shipped at ambient temperature.
- Upon kit arrival, Lysis Buffer Buccal Swab DNA (LB) should be stored at 2–8 °C. The other kit components are stable at room temperature (15–25 °C).
- Lysis Solution Buccal Swab (LS) and prepared buffer Lysis Buffer Buccal Swab (LB) may form precipitate upon storage below 8 °C. Before use, dissolve by allowing the buffer to warm up to room temperature for a minimum of 10 minutes and mix by inverting..

Before starting DNA extraction with the **EchoLUTION Buccal Swab DNA Kit (REF: 010-010-050 and REF: 010-010-250)**, prepare the following:

- Add the complete Lysis Solution Buccal Swab DNA (LS) to the Lysis Buffer Buccal Swab DNA (LB, brown bottle). Mix by inverting 5–10 times, then let stand for 10 minutes to reduce the foam. After mixing, mark the label on Lysis Buffer Buccal Swab (LB) indicating the addition of the Lysis Solution Buccal Swab (LS) and add the date. Prepared Lysis Buffer Buccal Swab (LB) will be stable for six months when stored at –20 °C. If storage is not possible at this temperature, the Lysis Buffer Buccal Swab (LB) can be stored at 2–8 °C for two weeks.

4.2. *Sample collection*

Buccal cells can be collected with a wide range of dry swabs. The EchoLUTION Buccal Swab DNA Kit is compatible with various swabs like Sarstedt® Forensic Swabs, Whatman® OmniSwabs, Copan dry eSwabs®, and Isohelix DNA/RNA Buccal Swabs (SK-1S, SK-3S) and RapiDri Swab (RD-01). The DNA yield from buccal samples depends on the amount and quality of cellular material on the swabs. Therefore, to guarantee a proper sample collection, we recommend the following:

- Refrain from eating, drinking, chewing gum, or brushing your teeth for at least half an hour before sample collection.
- Scrape the swab or brush against the inside of each cheek for 30 seconds.
- After sample collection, allow the swab to dry for at least one hour before processing the sample.
- Store swabs dry and at room temperature.

4.3. *Storage and stability of purified nucleic acids*

Purified nucleic acids can be stored at 2–8°C for one week maximum. For long-term storage of purified nucleic acids, it is recommended to store the DNA samples at –20 °C.

5. WARNINGS AND SAFETY INSTRUCTIONS

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please refer to the instructions supplied with our safety data sheets (SDS). Please contact us for the SDS.

Table3: ***EchoLUTION Buccal Swab DNA Kit safety information***

Component	Hazardous component	GHS symbol	Hazard statements	Precautionary statements	Additional statement
Lysis Buffer Buccal Swab (LB)	Tris (2-carboxyethyl) phosphine hydrochloride	 Danger	H314	P101; P102; P103; P260, P303+P361+P353, P305+P351+P338 P310; P405, P501	-
Clearing Solution Buccal Swab (CS)	Strontium chloride	 Danger	H318	P101; P102; P103; P280 P305+P351+P338 P310; P501	-

Hazard statements

H314:	Causes severe skin burns and eye damage.
H318:	Causes serious eye damage.

Precautionary statements

P101:	If medical advice is needed, have product container or label at hand.
P102:	Keep out of reach of children.
P103:	Read carefully and follow all instructions.
P260:	Do not breathe dusts or mists.
P280:	Wear eye protection/face protection.
P303+P361+P353:	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310:	Immediately call a POISON CENTER/doctor.
P405:	Store locked up.
P501:	Dispose of contents/container in accordance with local/regional/ national/international regulations.

6. DISPOSAL

Please follow local regulations regarding the collection and disposal of hazardous waste. Contact your waste disposal company to obtain information on laboratory waste disposal (waste code number 16 05 06). For further information, please refer to the instructions supplied with our SDS. Please contact us for the SDS.

Dispose biological samples and all liquid waste generated during the purification procedure as biohazardous waste.

A. Components and Spin Columns

No special measures for disposal are necessary. Components that have come into contact with potentially infectious material should be autoclaved. Used components may retain some buffer residues and should be disposed of according to local/regional/national/international regulations.

7. PROTOCOL

This protocol has been developed to extract total DNA from buccal cells from buccal swab samples using the EchoLUTION Buccal Swab DNA Kit.

This is not a silica-based kit. Please read the instructions carefully before starting!

Preparation before starting:

- Vortex EchoLUTION Spin Column thoroughly to homogenize the purification matrix and remove air bubbles. If necessary, flick or gently spin down by hand until it is free of air bubbles. Place each column in a 2 mL reaction tube (not provided) and let them stand to sediment the matrix for at least 10 minutes.

NOTE:

- *For improved sedimentation of the matrix, we recommend performing this step the day before and letting the columns stand overnight.*

- Lysis Solution Buccal Swab (LS) and prepared buffer Lysis Buffer Buccal Swab (LB) may form precipitate upon storage below 8 °C. Before use, dissolve by allowing the buffer to warm up to room temperature for a minimum of 10 minutes and mix by inverting.

- Before using the kit for the first time, prepare the Lysis Buffer Buccal Swab (LB) by adding the complete Lysis Solution Buccal Swab (LS) to the Lysis Buffer Buccal Swab (LB, brown bottle). Mix by inverting 5–10 times, then let stand for 10 minutes to reduce the foam. After mixing, mark the label on the Lysis Buffer Buccal Swab (LB) bottle to indicate the addition of the Lysis Solution Buccal Swab (LS) and add the date. Prepared Lysis Buffer Buccal Swab (LB) will be stable for six months when stored at –20 °C. Alternatively, the Lysis Buffer Buccal Swab (LB) can be stored at 2–8 °C for two weeks.

- Prepare 1.5 mL microcentrifuge tubes (not provided, preferably safe lock) for each sample.

- Pre-heat the thermal shaker to 80 °C.

- Set the microcentrifuge to 1,000 x *g*.

- Carry out the complete DNA extraction at room temperature.

IMPORTANT NOTE:

- *Choose x g (rcf), not rpm, unless stated otherwise.*
- *Make sure the Lysis Buffer Buccal Swab (LB) is prepared and warmed up to room temperature.*

1. *Spin Column preparation*

- Loosen the cap of the Spin Column by half a turn and snap off the bottom.
- Place the Spin Column in a 2 mL reaction tube (not provided).

- Centrifuge 1 minute at 1,000 x *g*, and discard flow-through.
- Place the Spin Column in a fresh 1.5 mL reaction tube (not provided).

ALTERNATIVLY:

You can use the BioEcho Cap Puncher to open the column (not supplied, product number: 050-001-001). To use the cap puncher correctly, punch a hole into the column cap and lift the column together with the cap puncher out of the 2 mL reaction tube. Snap off the bottom closure of the column and detach the cap puncher. Place the punched Spin Column back into the 2 mL reaction tube. Centrifuge 1 minute at 1,000 x g, discard flow-through and place the Spin Column in a fresh 1.5 mL reaction tube.

NOTE:

Proceed directly with step 2.

2. *Sample preparation and lysis*

- Prepare a 1.5 mL reaction tube and put the dry swab in it.
- Cut the head off the swab stick shortly above the swab end. Please make sure that the 1.5 mL reaction tube closes properly with the swab inside.
- Add 350 µL of prepared Lysis Buffer Buccal Swab (LB) to each sample.
- Close the reaction tube and incubate at 80 °C in a thermal shaker with constant shaking of 1,400 rpm for 2 minutes.

IMPORTANT NOTE:

- *Longer incubation can lead to DNA degradation. Therefore, it is highly important to not prolong the incubation period.*

NOTE:

- *Allow samples to cool down to room temperature for 10 minutes. If you want to speed up the process, you can place the samples on ice for 1 minute or at 4 °C for 5 minutes.*

3. DNA purification

- Add 30 µL of Clearing Solution Buccal Swab (CS) to the lysate and vortex shortly.
- Open the Spin Column and transfer 100 µL lysate to the prepared Spin Column.

IMPORTANT NOTE:

- *The use of wide-bore tips is recommended for mixing and transfer of the lysate to the Spin Column.*
- *Pipet slowly, drop-by-drop, and vertically onto the middle of the Spin Column to not destroy the matrix surface.*
- *Do not touch the matrix bed with the pipette tip during sample loading!*

NOTE:

- *If you have used the Cap Puncher, make sure that you pipette vertically through the hole in the lid. Do not punch the pipette tip into the matrix while loading the lysate onto the EchoLUTION Spin Column.*

- Close the cap of the Spin Column and loosen the cap again by a half turn.
- Centrifuge the loaded column for 1 minute at 1,000 x *g*.
- Purified DNA is in the flow-through and ready-to-use.

The extracted DNA can be used immediately or stored at 2–8 °C for one week maximum. For long-term storage, place your DNA samples at –20 °C.

IMPORTANT NOTE:

- *For spectrophotometric analysis, use the Low-TE Buffer supplied with the kit as blank.*

7.1. *Quick protocol EchoLUTION™ Buccal Swab DNA Kit: spin column kits*

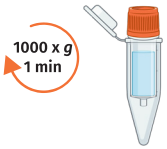
IMPORTANT NOTE: *Please use the quick protocol only after you have read and understood the complete user manual.*

Preparation before starting

- Vortex EchoLUTION Spin Columns thoroughly to homogenize the purification matrix. Then place them in a 2 mL reaction tube and let them stand for at least 10 minutes.
- Prepare the Lysis Buffer Buccal Swab (LB).
- Pre-heat the thermal shaker to 80 °C.
- Set the microcentrifuge to 1,000 x *g*.

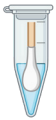
1. *Spin Column preparation*

- Loosen the cap of the Spin Column half a turn and snap off the bottom.



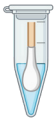
- Place Spin Column in 2 mL reaction tube.
- Centrifuge 1 min at 1,000 x *g*.
- Discard flow-through.
- Place Spin Column in a new reaction tube.

2. *Sample preparation and lysis*

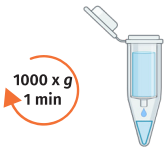


- Place dry swab in a reaction tube.
- Add 350 µL LB.
- Incubate at 80 °C for 2 min at 1,400 rpm.
  IMPORTANT NOTE: *Longer incubation can lead to DNA degradation. Therefore, it is highly important to not prolong the incubation period.*
- Cool down at room temperature for 10 min.

3. *DNA purification*



- Add 30 µL CS.
- Vortex shortly.



- Transfer 100 µL lysate. Pipet slowly, drop-by-drop onto the middle of the column without touching the matrix.
- Centrifuge 1 min at 1,000 x *g*.
- Purified DNA is in the flow through and ready-to-use.

8. QUALITY CONTROL

Following the BioEcho Quality Management System, each lot of the EchoLUTION Buccal Swab DNA Kit is tested against predetermined specifications to ensure consistent product quality.

The Certificate of Analysis (CoA) can be requested by contacting QA@bioecho.de.

9. TROUBLESHOOTING

Observation	Comments and suggestions
DNA degradation	**Incorrect lysis collection** During the lysis step it is important to stick to time and temperature mentioned in the protocol, as longer lysis time, or elevated temperatures can lead to degradation of the DNA.
DNA yield and concentration is low	**Incorrect sample collection** The DNA yield depends on the sample collection. Therefore, we recommend following the sample collection recommendations described in section 4.2. **Loading of purification matrix** The correct loading of the Spin Column is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the Spin Column to not destroy the matrix surface. Do not touch the matrix bed with the pipette tip during sample loading. **Centrifuge settings** Most centrifuges offer the choice between rpm and *g*-force (rcf); if rcf is not available, calculate rpm (see section 2.2). Always make sure to use the correct time mentioned in the protocol to avoid insufficient passage through the matrix bed.

DNA yield and concentration is low (continuation)

Highly tilted matrix in column

A highly tilted matrix after conditioning can lead to insufficient time of interaction with the matrix, which can result in a poor extraction performance. Please read observations "Highly tilted matrix in column" for further instructions.

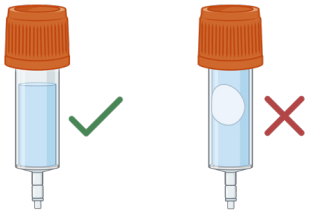
Air bubbles in Spin Column matrix

Inappropriate homogenization

To homogenize the matrix in the Spin Columns, the Spin Columns need to be vortexed thoroughly. You can perform the vortexing in an upright position, on the side, or upside down depending on what works best for you. It can happen, especially in an upside-down position, that air bubbles are introduced (see picture below), but for a good performance, it is essential to remove these air bubbles.

To remove air bubbles, flick or gently spin down by hand until it is free of air bubbles or quickly vortex again in an upright position at the end. Place each column in a 2 mL reaction tube (not provided) and let them stand to sediment the matrix until used.

For improved sedimentation of the matrix, we recommend that this step is performed upon receipt of the kit and to store them in an upright position.



Highly tilted matrix in column

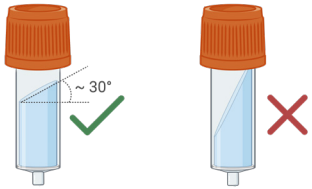
Inappropriate handling of Spin Column

A highly tilted matrix after conditioning leads to insufficient interaction time between the DNA and the matrix, which can result in poor extraction performance. However, when using fixed-angle rotor centrifuges, the matrix does not become fully horizontal after conditioning. A slightly tilted

Highly tilted matrix in column (continuation)

surface according to the angle of the rotor (usually 30°) is to be expected, and this does not limit the purification ability.

But if you observe that the matrix is tilted to a higher degree (see picture below), we recommend prolonging the standing time after vortexing up to overnight before conditioning. For quicker processing, we recommend vortexing the Spin Columns upon receipt of the kit and storing them in 2 mL microcentrifuge tubes in an upright position till used. See section 2.2 for further instructions.



Another reason could be that the Spin Column was completely closed during centrifugation and a vacuum was generated. Alternatively, if you want to avoid prolonging the standing time, we suggest using a swing-out rotor centrifuge with our Spin Column Adapter for Plate Centrifuges (product number: 050-011-024).

A_{260}/A_{280} and A_{260}/A_{230} values are low

Wrong blank in measurements
Use supplied Low-TE Buffer (T) as blank for measurements.

Incorrect lysate volume
Avoid overloading the purification matrix by increasing lysate volume. Using a higher volume than the one recommended in the protocol will compromise the sample purity.

Poor performance in downstream experiments

Incorrect lysis conditions
During the lysis step it is important to stick to time and temperature mentioned in the protocol, as longer lysis time, or elevated temperatures can lead to degradation of the DNA.

Inappropriate handling of Spin Column
The Spin Column was closed during centrifugation, and a vacuum was generated. If you observed that the matrix is tilted and not flat after centrifugation, we recommend prolonging the standing time to 30 minutes before conditioning. For quicker processing, we recommend vortexing the Spin Columns upon receipt of the kit and storing them in 2 mL microcentrifuge tubes in upright position till used. See section 2.2 for further instructions.

Alternatively, if you want to avoid prolonging the standing time, we suggest using a swing-out rotor centrifuge with our Spin Column Adapter for Plate Centrifuges (product number: 050-011-024).

Loading of purification matrix
The correct loading of the Spin Column is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the Spin Column to not destroy the matrix surface. Do not touch the matrix bed with the pipette tip during sample loading.

Centrifuge settings
Most centrifuges offer the choice between rpm and *g*-force (rcf); if rcf is not available, calculate rpm (see section 2.2). Always make sure to use the correct time mentioned in the protocol to avoid insufficient passage through the matrix bed.

Occurrence of cross-contamination	**Contaminated pipettes** The use of contaminated pipettes can lead to cross-contamination. We recommend using a separate set of pipettes for sample preparation and PCR preparation. These pipettes should be cleaned thoroughly at regular intervals.
Eluate is missing or volume to low	**Inappropriate handling of Spin Column** The Spin Column was closed during centrifugation, and a vacuum was generated. See section 2.2 for further instructions. **Centrifuge settings** Most centrifuges offer the choice between rpm and *g*-force (rcf); if rcf is not available, calculate rpm (see section 2.2). Always make sure to use the correct time mentioned in the protocol to avoid insufficient passage through the matrix bed.

For questions and further troubleshooting, please contact us!

10. LIMITATIONS OF USE

Limitations regarding EchoLUTION Buccal Swab DNA Kit are listed below:

- Strict compliance with the user manual is required for DNA purification. Following good laboratory practices is crucial for the successful use of the product. Appropriate handling of the reagents is essential to avoid contamination and impurities.
- The DNA yield varies and is dependent on several factors including the technique of the person taking the sample.
- The proof of principle for the EchoLUTION Buccal Swab DNA Kit was evaluated and confirmed using state-of-the-art qPCR. Performance parameters are highly dependent on the quality of sample collection.
- The kit is for research use only.

11. SYMBOLS

The following table describes the symbols that appear on the labeling of the EchoLUTION Buccal Swab DNA products and in this user manual.

Table 4: ***EchoLUTION Buccal Swab DNA Kit symbols.***

Symbols	Description
	Manufacturer
	For research use only
	Product number
	Batch code
	Contains sufficient for < n > reactions
	Temperature limitation
	Do not re-use
	Expiration date
	Consult instructions for use

WE ARE INTERESTED IN YOUR EXPERIENCE WITH BIOECHO PRODUCTS!

With questions or suggestions or for further troubleshooting, please contact us.

Visit our website and shop for further information, tutorials and application notes.

This user manual can be found in our shop on the corresponding product page.

Interested in publishing an application note with us? Please get in touch!

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