EchoLUTION™ Buccal Swab DNA Kit

96-well plate kits

USER MANUAL

RUO

REF

010-110-002

010-110-008

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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 5 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*

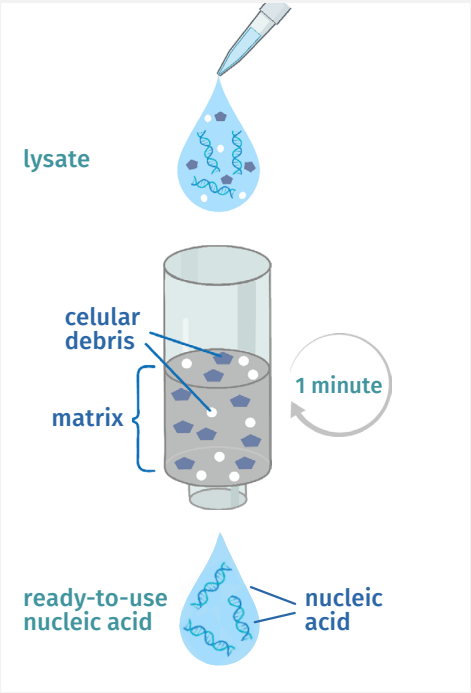
All BioEcho nucleic acid extraction kits are based on the EchoLUTION technology, which consists of tailored sample processing that includes lysis followed by single-step purification. The purification step works differently than conventional methods such as magnetic-bead and silica kits based on the 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 Purification Plate 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 Purification Plate, 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, 96-well plate format***

Product number	010-110-002	010-110-008
Product name	EchoLUTION Buccal Swab DNA Kit (2 × 96)	EchoLUTION Buccal Swab DNA Kit (8 × 96)
Reactions	192	768
Lysis Buffer Buccal Swab* (LB)	2 × 700 mg	8 × 700 mg
Lysis Solution Buccal Swab* (LS)	2 × 50 mL	8 × 50 mL
Clearing Solution Buccal Swab (CS)	1 × 10 mL	1 × 40 mL
Low-TE Buffer (T)	1 × 1.2 mL	2 × 1.2 mL
Purification Plate 96 Type 1	2 plates	8 plates
Elution Plate 96 Type 1	2 plates	8 plates
Lysis Plate Type 4	2 plates	8 plates
Adhesive Foil	2 foils	8 foils

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

3.2. Materials required but not provided

A. Conditioning Plate

The Conditioning Plate is necessary to remove the matrix storage buffer from the Purification Plate. The Conditioning Plate can be reused up to 20 times and needs to be ordered separately. To purchase this item, use the product number 060-001-002 or 060-001-008, depending on the number of plates required.

B. Multichannel reagent reservoir

These reservoirs are necessary when using multichannel pipettes for transferring prepared master mixes.

C. Plates for counterbalance in centrifuge

In case an odd number of plates is processed, prepare an additional plate stack to ensure the centrifuge is balanced and fill the wells with the appropriate amount of water.

3.3. Laboratory equipment needed

A. Plate centrifuge

For the procedure, plate centrifuges with the following specifications are mandatory:

- Standardized Society for Biomolecular Screening (SBS) format
- Capable of at least 1,000 x *g*
- At least 5 cm clearance for plate holder height
- Swing-out rotor

B. Pipetting equipment

Pipetting can be performed using a single-channel pipette as well as a multi-channel pipette for 200 µL up to 1,000 µL. We recommend using wide-bore tips for mixing and transferring the lysate to the purification matrix.

C. Thermal shaker for plates

The thermal shaker is used for the lysis step. It needs to reach up to 80 °C and 1,400 rpm (e.g., Eppendorf® ThermoMixer® C). Alternatively, you can use a heating block or heat chamber.

D. 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 and 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: 010-110-002 and REF: 010-110-008)**, 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 Purification Plates

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:

- 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. *Purification Plate preparation*

- Detach first the lower and then the upper foil from the Purification Plate. Be sure to keep the plates in a horizontal position while removing the foils, as the wells contain liquid.
- Place the Purification Plate on top of the Conditioning Plate (not provided, product number: 060-001-002 or 060-001-008).

- Centrifuge 1 minute at 1,000 x *g*, and discard flow-through.
- Place the Purification Plate on top of the Elution Plate.
- Proceed directly with step 2.

NOTES:

- *The centrifuge rotor should be capable of holding plate sandwiches that have a height of 5 cm.*
- *Conditioning Plates can be reused.*
- *If the Purification Plate was not shipped or stored upright, the resin may stick to the upper foil. In this case, shake plate until the resin is removed from upper foil.*
- *Make sure the foil is completely removed from the bottom.*

2. *Sample preparation and lysis*

- Load the Lysis Plate with one swab per well. Cut the heads off the swab sticks shortly above the swab ends. Do not cut the swab higher than the Lysis Plate height.
- Add 500 µL of the freshly prepared Lysis Buffer Buccal Swab (LB) to each well.
- Attach the Adhesive Foil to the Lysis Plate.
- Incubate the plate at 80 °C in a thermal shaker with constant shaking between 1,400 rpm for 5 min.

IMPORTANT NOTE:

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

NOTES:

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

3. DNA purification

- Remove the foil of the Lysis Plate and add 50 µL Clearing Solution Buccal Swab (CS) to each sample.
- To mix, set the pipet to a volume of 100 µL and mix by pipetting three times up and down.
- Transfer 100 µL lysate to the Purification Plate.

  IMPORTANT NOTE:

  - *The use of wide-bore tips is recommended for mixing and transfer of the lysate to the Purification Plate. Pipet slowly, drop-by-drop, and vertically onto the middle of the wells to not destroy the matrix surface (use an 8-channel pipette or robot).*
  - *Do not touch the matrix bed with the pipette tip during sample loading!*

- Centrifuge the plate stack (Purification Plate on top of the Elution Plate) for 1 minute at 1,000 x *g*.
- Purified DNA is in the flow-through and ready-to-use.

  NOTE:

  - *The supplied Adhesive Foil cannot be used for the storage of nucleic acids.*

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.*

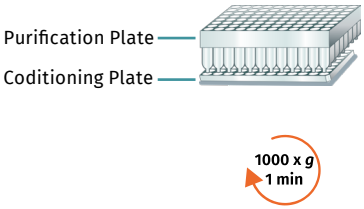
7.1. Quick protocol EchoLUTION™ Buccal Swab Kit: 96-well plate kits

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

Preparation before starting

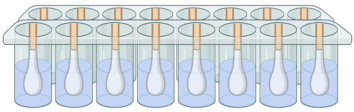
- Prepare the Lysis Buffer Buccal Swab (LB).
- Pre-heat the thermal shaker to 80 °C.
- Set the microcentrifuge to 1,000 x *g*.

1. Purification Plate preparation



- Detach first the lower and then the upper foil from the Purification Plate.
- Place the Purification Plate on top of the Conditioning Plate.
- Centrifuge plate stack for 1 min at 1,000 x *g*.
- Discard the flow-through.
- Place the Purification Plate on top of the Elution Plate.

2. Sample preparation and lysis

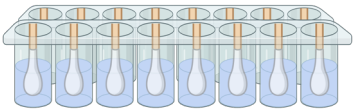


- Load the Lysis Plate with one swab per well. Cut the heads off the swab sticks shortly above the swab ends.
- Add 500 µL LB.
- Attach Adhesive Foil and incubate at 80 °C for 5 min at 1,400 rpm.

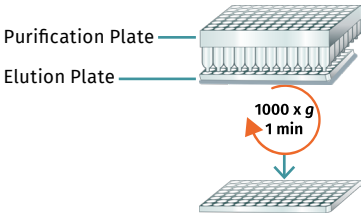
IMPORTANT NOTE: *Longer incubation can lead to DNA degradation. Therefore, it is highly important to not prolong the incubation period.*

- Cool down at room temperature for 10 min.

3. DNA purification



- Add 50 µL CS.
- Set pipette volume to 100 µL and mix by pipetting three times up and down.



- Transfer 100 µL lysate. Pipet slowly, drop-by-drop onto the middle of the wells without touching the matrix.
- Centrifuge plate stack for 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 matrix is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the purification matrix to not destroy the 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.

A_{260}/A_{280} and A_{260}/A_{230} values are low	**Wrong blank in measurements** Use supplied Low-TE Buffer (T) as blank in 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. **Loading of purification matrix** The correct loading of the matrix is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the purification matrix to not destroy the 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 not available, calculate the rpm see section 2.2. Always make sure to stick to 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. BioEcho recommends a separate set of pipettes for sample preparation and PCR preparation, which should be cleaned thoroughly at regular intervals.
Eluate is missing or volume to low	**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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