EchoLUTION™ Viral RNA/DNA Kit

Plate kits

USER MANUAL

CE IVD

REF

012-051-002-Dx

012-051-008-Dx

012-051-016-Dx

012-102-002-Dx

012-102-008-Dx

012-102-016-Dx

BioEcho Life Sciences GmbH | BioCampus Cologne, Main Building | Nattermannallee 1 | 50829 Cologne, Germany

TABLE OF CONTENTS

1. INTENDED PURPOSE

The EchoLUTION Viral RNA/DNA Kit is designed for reliable extraction of viral RNA or DNA and serves as an accessory for subsequent in vitro diagnostic analysis. The EchoLUTION technology is based on a tailored lysis step followed by nucleic acid purification in a single centrifugation step. Impurities are held back by the purification matrix while the viral RNA and DNA flow through untouched. With the product, viral nucleic acids from nasopharyngeal swabs, oropharyngeal swabs, or stool samples can be extracted. The product is intended to be used by laboratory professionals with experience in molecular biotechnology techniques, especially in the extraction of nucleic acids and handling of pathogenic material.

2. EXPLANATION OF THE KIT

The product is an FDA-regulated, CE-marked, EU 2017/746 (IVDR)-compliant nucleic acid extraction kit.

The EchoLUTION Viral RNA/DNA Kit is characterized by the EchoLUTION single-step purification technology, an instant lysis without incubation for swab samples, and a fast lysis step of 10 minutes for stool samples. Together, the overall extraction time is reduced to a minimum and the workflow results in consistent performance.

In connection with appropriate IVD-downstream applications (e.g., RT-qPCR) the kit enables fast and reliable detection of viral infections.

The EchoLUTION Viral RNA/DNA Kit benefits are:

- Short processing time
- Lysis of swab samples without incubation
- Few protocol steps
- High sample throughput with minor equipment and capital investment
- Up to 70 % less plastic waste

For further details about kit specifications, see Table 1.

Table 1: ***Kit specifications***

Specification	Description
Sample input	Respiratory and enteropathogenic viruses
Sample type	Naso-, oropharyngeal swabs, and stool samples
Sample condition	Fresh, dry, or in transport media swabs. Frozen or fresh (stool samples)
Purified nucleic acid	Viral RNA or DNA
Elution volume	90 µL

2.1. *Single-step purification principle*

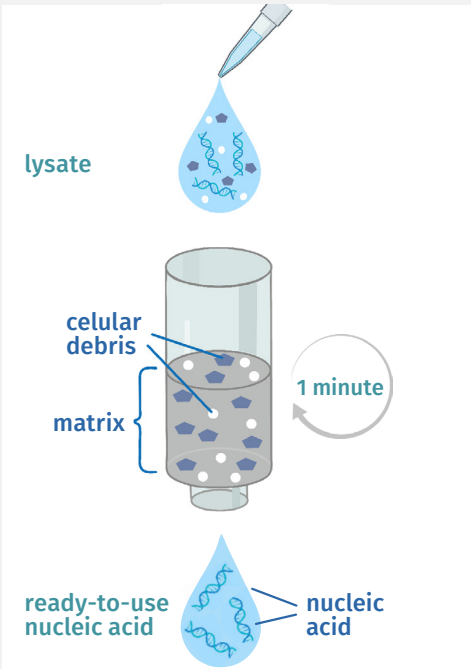
All BioEcho nucleic acid extraction kits are based on the EchoLUTION technology, which consists of tailored sample processing that includes lysis followed by single-step purification. The purification step works differently than conventional methods such as magnetic-bead and silica kits (based on the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

The lysate is transferred onto the 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 adsorption. 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. Due to the repeated washing steps, silica-based methods are time-consuming, labor-intensive, and environmentally unfriendly.

Handling of 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.

Automation of the extraction

Some customers have already established the EchoLUTION Viral RNA/DNA Kit on automated liquid handling systems. These systems include Tecan, Hamilton, Perkin Elmer, or Flow Robotics. Some of the mentioned companies even offer full automation, including barcode recognition and built-in centrifugation. We will be happy to work with you and a system's manufacturer to develop an automation solution tailored to your needs. Please contact us for further information.

Handling DNA

In general, cautious microbiological practices should always be used when working with DNA. The most common sources of contamination are dust and hands, as they can hold e.g., 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 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.

Handling RNA

For high-quality RNA extraction, the samples must be free of ribonucleases (RNases), as these enzymes digest RNA very efficiently. In general, RNases are very active, stable, and difficult to inactivate. Even small amounts can digest sufficient RNA to drastically reduce the yield. To avoid RNase contamination of your samples, some things need to be considered. Always eliminate possible RNase contamination from any consumables you will use before starting your extractions. Make sure that the working area is free of RNases throughout the whole procedure to avoid introducing RNases into the samples.

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 RNase-removing solutions.

In general, working cautiously using sterile microbiological practices should always be used when working with RNA to avoid contamination risk. The most common sources of RNases 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 RNA samples. Replace gloves regularly and keep tubes closed when possible.

For long-term storage, purified RNA can be stored at –70 °C. Samples can be aliquoted to avoid repeated thaw and freeze cycles. When working with the RNA for downstream applications, thaw on ice and keep on ice while working.

Input material

For optimal results, it is vital to use the correct amount of input material to achieve best DNA and RNA purity and yield.
Factors that influence the DNA and RNA yield are:

- Different swab types can have different virus load. Furthermore, stool samples may vary a lot, also within one sample, in viral content.
- Storage time and storage conditions may influence the quality of your sample. It should be considered that virus material is subject to degradation under certain circumstances due to environmental influences (direct sunlight, freezing the sample, high temperatures).
- The volume of transport media will directly influence the concentration of viral particles in your sample. Do not use too much or too little transport medium. The swab should be covered with medium

Lysis

Incorrect volume of lysis buffer leads to low DNA and RNA recovery.

In general, but especially for RNA/DNA extraction from stool samples, it is important to stick the correct time and temperature mentioned in the protocol for the lysis step, as shorter lysis can lead to an inefficient lysis of the sample and a longer lysis time can lead to degradation of the RNA or DNA.

Furthermore, it is crucial to use the recommended devices and accessories mentioned in section 3 (Materials).

3. MATERIALS

3.1. Materials provided

Table 2: ***Content of EchoLUTION Viral RNA/DNA Kit, 48-well plate format***

Product number	012-051-002-Dx	012-051-008-Dx	012-051-016-Dx
Product name	EchoLUTION Viral RNA/DNA Kit (2 × 48)	EchoLUTION Viral RNA/DNA Kit (8 × 48)	EchoLUTION Viral RNA/DNA Kit (16 × 48)
Reactions	96	384	768
Purification Plate 48 Type 2	2 plates	8 plates	16 plates
Purification Plate 96 Type 2	—	—	—
Elution Plate 96 Type 1	2 plates	8 plates	16 plates
LyseNtact Buffer New Formula	1 × 5 mL	1 × 20 mL	2 × 20 mL

Table 3: ***Content of EchoLUTION Viral RNA/DNA Kit, 96 -well plate format***

Product number	012-102-002-Dx	012-102-008-Dx	012-102-016-Dx
Product name	EchoLUTION Viral RNA/DNA Kit (2 × 96)	EchoLUTION Viral RNA/DNA Kit (8 × 96)	EchoLUTION Viral RNA/DNA Kit (16 × 96)
Reactions	192	768	1536
Purification Plate 48 Type 2	—	—	—
Purification Plate 96 Type 2	2 plates	8 plates	16 plates
Elution Plate 96 Type 1	2 plates	8 plates	16 plates
LyseNtact Buffer New Formula	1 × 10 mL	1 × 39 mL	2 × 39 mL

3.2 Materials required but not provided

A. Lysis Plate

The Lysis Plate necessary for mixing the LyseNtact Buffer New Formula with the swabs is not included in the kit. For nasopharyngeal swabs and oropharyngeal swabs please use Lysis Plate 96 Type 2 (product number 060-004-008-Dx).

For lysis of stool samples please use the Eppendorf® Deepwell Plate 96/1000 µL in combination with the Eppendorf ThermoMixer® C and the heating block Eppendorf SmartBlock™ DWP 1000.

The use of other thermal shaker devices and lysis plates must be validated in combination with the kit before use, because they might not be able to heat the samples efficiently. If you have questions regarding the validation of your device, please contact us.

B. Conditioning Plate

The Conditioning Plate is necessary to remove the matrix storage buffer from the Purification Plate. The Conditioning Plate can be re-used up to 20 times. Please remove the collected buffer after every use. Please do not use any alternative plate. The plate needs to be ordered separately. To purchase this item, use the product number 060-001-002-Dx or 060-001-008-Dx.

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.

D. Microcentrifuge tubes (for stool samples only)

Use a 1.5 mL or 2 mL tube to resuspend the stool sample in buffer.

E. Adhesive Foil (for stool samples only)

The Adhesive Foil is recommended for lysis of stool samples. It is an air-permeable foil, which is used to seal the Lysis Plate during the 10-minute heating step of the lysis. To order the foil, please use the product number 050-007-008-Dx or 050-007-050-Dx.

3.3. Optional materials

A. LyseNtact Buffer New Formula (39 mL)

Additional LyseNTact Buffer New Formula is needed for the alternative protocol of dry nasopharyngeal or oropharyngeal swabs described in chapter 7, section 2.C (Alternative protocol to achieve higher concentration of nucleic acids: Dry nasopharyngeal swabs and dry oropharyngeal swabs). This protocol has been developed to achieve higher concentrations of nucleic acids by skipping the 1:1 dilution of the transport medium with the LyseNtact Buffer New Formula. Use this protocol if higher concentrations of viral nucleic acids or lower Ct values are of high importance for your assay. The LyseNtact Buffer New Formula provided with the kit is not sufficient for this protocol. Please order additional buffer using the product number 012-112-039-Dx. One bottle is sufficient for 39 samples.

3.4. Laboratory equipment needed

A. Safety cabinet

Handling of potentially infectious samples must follow local/regional/national regulations. Pipetting and processing of potentially infectious material must be carried out under a designated class 1 safety cabinet or in a comparable facility for personal protection.

Activities that are likely to involve a bioaerosol hazard must be performed in a safety microbiological cabinet (MSC) or a comparable facility for personal protection (e.g., fume high-efficiency particulate air filter).

B. 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 × *g*
- Capable of holding plate stacks of 5 cm height
- Swing-out rotor

C. 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. Pipette tips with filters should always be used for pipetting infectious samples. A separate pipette should be used for the positive control: Positive controls must never be pipetted with the same pipette as the samples.

D. Thermal shaker for plates (for stool samples only)

The thermal shaker is used for the lysis step of stool samples. Please only use the Eppendorf ThermoMixer C with the adapter Eppendorf SmartBlock DWP 1000 and the lysis plate Eppendorf Deepwell Plate 96/1000 µL.
Other thermal shaker devices and lysis plates must be validated in combination with the kit before use, because they might not be able to heat the sample efficiently. If you have questions regarding the validation of your device, please contact us.

E. Vortex mixer

A vortex mixer is required for homogenization of stool samples and vortexing swab samples in media.

F. Benchtop centrifuge (for stool samples only)

A benchtop centrifuge is necessary for the preparation of stool samples. The centrifuge should be able to accommodate 1.5 or 2 mL reaction tubes and be capable of a centrifugation speed of 10,000 × *g*.

G. Inoculation loops (for stool samples only)

Inoculation loops are required to transfer the primary stool material into 1 mL TE Buffer or PBS Buffer. We recommend inoculation loops from Sarstedt® (inoculation loop, 10 µL, PS, blue, sterile) to transfer 25 mg of stool sample. Due to handling differences, please check beforehand that the correct amount of stool is transferred (approximately 25 mg).

4. STORAGE AND STABILITY

4.1. Kit and reagents

- The EchoLUTION Viral RNA/DNA Kit is shipped at ambient temperature.
- Upon kit arrival, the Purification Plates and the LyseNtact Buffer New Formula (LB, brown bottle) should be stored at 2–8 °C. The other kit components are stable at room temperature (15–25 °C).
- The Purification Plates need to be stored as described on the label (label facing up).

Table 4: ***Stability of EchoLUTION Viral RNA/DNA Kit components***

Component	Stability
LyseNtact Buffer New Formula	Unopened stable at 2–8 °C for a minimum of 12 months until expiration date mentioned on the label; upon opening the bottle, stable at 2–8 °C for 6 months
Purification Plate 96 Type 2	Unopened stable at 2–8 °C for a minimum of 12 months until expiration date mentioned on the label
Elution Plate 96 Type 1	Stable at 2–25 °C until expiration date mentioned on label

4.2. Sample collection

Nasopharyngeal swabs and oropharyngeal swabs can be collected with different swab types and used fresh, stored dry, or in transport media. Table 12 in chapter 9 (Analytical performance) summarizes the transport media and resuspension media that are compatible and have been successfully tested with the EchoLUTION Viral RNA/DNA Kit.
Other media need to be validated before use. To use transport media not listed in table 9, please contact us for support.
The DNA and RNA yield from swab samples depends highly on the amount of viral material on the swabs and the used swab types. Therefore, use swab samples according to the manufacturer's recommendations and follow their instructions on transportation and storage.

4.3. Storage and stability of purified nucleic acids

For long-term storage of purified RNA, it is recommended storing the RNA samples at –70 °C. Avoid repeated thaw and freezing cycles, aliquot RNA if needed.
For long-term storage of purified DNA, it is recommended to store the DNA samples at –20 °C.

5. WARNINGS AND SAFETY INSTRUCTIONS

5.1. Reporting of incidents

The kit is intended for in-vitro diagnostic use and is therefore involved in diagnosis of diseases. Any serious incident that has occurred in relation to the EchoLUTION Viral RNA/DNA Kit needs to be reported to the manufacturer and the competent authority of the state in which the user and/or the patient is established. Please contact us immediately (see last page).

5.2. Hazardous and precautionary statements

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.

Table 5: ***EchoLUTION Viral RNA/DNA Kit safety information***

Component	Hazardous component	GHS symbol	Hazard statements	Precautionary statements	Additional statement
LyseNtact Buffer New Formula (LB)	Guanidinium Thiocyanate (R*,R*)-1,4-dimercaptobutane-2,3-diol Alcohols, C12-14-secondary, ethoxylated	 GH505 GH507 Danger	H302+H332; H314; H412	P101; P102; P103; P260; P303+P361+P353; P305+P351+P338; P310; P405; P501	EUH032

Hazard statements

H302+H332: Harmful if swallowed or if inhaled.
H314: Causes severe skin burns and eye damage.
H412: Harmful to aquatic life with long lasting effects.

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.
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.

Additional information

EUH032: Contact with acids liberates very toxic gas.

6. DISPOSAL

Please follow local regulations regarding the collection and disposal of hazardous waste and 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 of biological samples as biohazardous waste and all remaining liquid waste generated during the purification procedure according to local regulations.

A. LyseNtact Buffer New Formula

Harmful to aquatic life with long-lasting effects. Discharge into the environment must be avoided. Do not allow undiluted or large quantities of the product to reach groundwater, watercourse, or sewage system. Empty bottles may retain some buffer residues. Therefore, guarantee the dispose of contents/container under local/regional/national/international regulations.

B. Components and Purification Plates

Components that have come into contact with potentially infectious material should be autoclaved. Used components may retain some buffer residues, which should be disposed according to local/regional/national/international regulations.
The storage buffer of the Purification Plates collected in the Conditioning Plate during the conditioning step can be disposed of in the sink or on a tissue.

7. PROTOCOL

This protocol has been developed to extract viral RNA or DNA from nasopharyngeal swabs, oropharyngeal swabs, or stool samples using the EchoLUTION Viral RNA/DNA Kit.

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

Preparation before starting:

- Set plate centrifuge to 1,000 × *g*.
- Additionally for stool samples:
  - Set benchtop centrifuge to 10,000 × *g*.
  - Pre-heat the thermal shaker to 95 °C.

IMPORTANT NOTE:

- *Choose x g (rcf), not rpm, unless stated otherwise.*

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-Dx or 060-001-008-Dx).

- Centrifuge plate stack for 1 minute at 1,000 × *g*, discard flow-through.
- Place the Purification Plate on top of the Elution Plate.
- Proceed directly with step 2 (Sample preparation and lysis).

NOTES:

- *The centrifuge rotor should be capable of holding plate stacks that have a height of 5 cm.*
- *Conditioning Plates can be reused up to 20 times.*
- *Make sure the foil is completely removed from the bottom.*

2. *Sample preparation and lysis*

a. *Nasopharyngeal swabs and oropharyngeal swabs in transport media*

- Add 50 µL of LyseNtact Buffer New Formula to each well of the Lysis Plate (not provided, product number 060-004-008-Dx).

NOTE:

- *If using an Internal Control (IC) or in-process control (IPC), follow the manufacturer's instructions for using the IC/IPC. It can be added in this step. Any IC/IPC that is added before the purification step must be >500 nucleotides. We do not provide an IC/IPC.*

- Carefully vortex the swab in transport media.

- Add 50 µL of swab media (see Table 12 for more information) to each well of the prepared Lysis Plate. Mix by pipetting up and down at least three times.
- Continue with step 3 (DNA or RNA purification).

b. Dry nasopharyngeal swabs and dry oropharyngeal swabs

- Add 50 µL of LyseNtact Buffer New Formula to each well of the Lysis Plate (not provided, product number 060-004-008-Dx).
- Rinse the swab in 1 mL of transport or resuspension medium, e.g., Cobas® PCR Media (Uni Swab Sample Kit), LMS-Swab Amies, or PBS, to dissolve the viral particles.
- Carefully vortex the sample and incubate for 15 minutes at room temperature.
- Add 50 µL of the resuspended sample to each well in the prepared Lysis Plate. Mix by pipetting up and down at least three times.
- Continue with step 3 (DNA or RNA purification).

c. Alternative protocol to achieve higher concentration of nucleic acids: Dry nasopharyngeal swabs and dry oropharyngeal swabs

NOTE:

- *This protocol has been developed to achieve higher concentrations of viral nucleic acids by skipping the 1:1 dilution of the transport medium with the LyseNtact Buffer New Formula. Use this protocol if lower Ct values are of high importance for your assay. Please note that the LyseNtact Buffer New Formula provided within the kit is not sufficient for this protocol. Please order enough buffer (product number 012-112-039-Dx).*

- Rinse the swab in 1 mL of LyseNtact Buffer New Formula to dissolve the viral particles.
- Carefully vortex the sample and incubate for 15 minutes at room temperature.
- Continue with step 3 (DNA or RNA purification).

d. Stool samples

- Add 50 µL of LyseNtact Buffer New Formula to the Lysis Plate (not provided, Eppendorf Deepwell Plate 96/1000 µL).
- Resuspend approximately 25 mg primary stool sample in a 1.5 or 2 mL reaction tube (not provided) containing 1 mL TE Buffer or PBS Buffer. Gently vortex the reaction tube until the stool sample is resuspended.

NOTE:

- *We recommend inoculation loops to transfer the primary stool sample. Please see section 3.4 (Laboratory equipment needed) for further information. Overloading the sample with primary stool may lead to inhibition in the downstream assay.*

- Centrifuge the reaction tube for at least 15 seconds at 10,000 × *g* in a benchtop centrifuge to pellet the solid sample particles.
- Add 50 µL of the resuspended sample to each well in the prepared Lysis Plate. Mix by pipetting up and down at least three times.
- Attach Adhesive Foil (not provided, product number 050-007-008-Dx or 050-007-050-Dx) to the Lysis Plate.
- Incubate the Lysis Plate in the pre-heated thermal shaker at 95 °C with constant shaking at 800 rpm for 10 minutes.

  IMPORTANT NOTE:

  - *Please only use the Eppendorf ThermoMixer C with the adapter Eppendorf SmartBlockDWP 1000 and the lysis plate Eppendorf Deepwell Plate 96/1000 µL. Other thermal shaker devices and lysis plates must be validated in combination with the kit before use, because they might not be able to heat the sample efficiently.*

- Let Lysis Plate cool down for 5 minutes at room temperature and carefully remove Adhesive Foil before continuing with step 3 (DNA or RNA purification).

3. DNA or RNA purification

- Transfer 90 µL sample mixture to each well of the Purification Plate.

  IMPORTANT NOTE:

  - *Pipet slowly, drop-by-drop, and vertically onto the middle of the wells to not destroy the matrix surface (use of an 8-channel pipette is recommended).*
  - *Do not touch the matrix bed with the pipette tip during sample loading!*

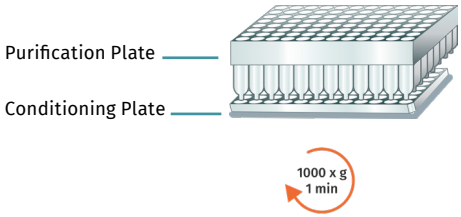
- *Centrifuge the plate stack (Purification Plate on top of the Elution Plate) for 1 minute at 1,000 × g.*
- *Purified DNA or RNA is in the flow-through and ready-to-use.*

The extracted DNA can be used immediately or stored. For long-term storage place your DNA samples at –20 °C.
The extracted RNA can be stored or used directly. For long-term storage place your RNA samples at –70 °C.

7.1. *Quick protocol EchoLUTION™ Viral RNA/DNA Kit: 96-well plate kits*

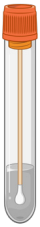
IMPORTANT NOTE: *Please use the quick protocol only after you have read and understood the complete user manual.*

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



Swabs in Medium

- Add 50 µL of LyseNtact Buffer New Formula to each well of Lysis Plate.
- Vortex swab in medium.
- Add 50 µL of transport medium to Lysis Plate and mix by pipetting up and down.

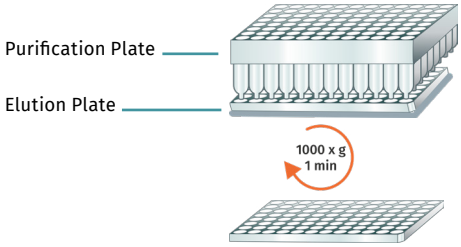
Dry swabs

- Add 50 µL of LyseNtact Buffer New Formula to each well of Lysis Plate.
- Rinse swab in 1 mL medium.
- Vortex sample and incubate 15 min at RT.
- Add 50 µL of medium to lysis plate and mix by pipetting up and down.

Stool samples

- Add 50 µL of LyseNtact Buffer New Formula to each well of Lysis Plate.
- Mix approx. 25 mg stool with 1 mL TE buffer or PBS buffer.
- Vortex until stool sample is resuspended.
- Let stand on ice until next step.
- Centrifuge for 15 s at 10,000 x *g*.
- Transfer 50 µL of supernatant to lysis plate and mix by pipetting up and down.
- Attach Adhesive Foil to the Lysis Plate.
- Incubate Lysis Plate for 10 minutes at 95 °C.
- Let Lysis Plate cool down at RT for 5 minutes.

3. DNA or RNA purification



- Transfer 90 µL lysate to the Purification Plate. Pipet slowly, drop-by-drop onto the middle of the column without touching the matrix.
- Centrifuge plate stack for 1 min at 1,000 x *g*.
- Purified DNA or RNA is in the flow-through.

8. QUALITY CONTROL

Following the BioEcho Quality Management System, each lot of the EchoLUTION Viral RNA/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. ANALYTICAL PERFORMANCE

9.1. Experimental setup

The EchoLUTION Viral RNA/DNA Kit was used according to the manufacturer's instructions for isolation and purification of viral RNA/DNA from biological specimens for in vitro diagnostic use.

The data exemplarily show a SARS-CoV-2 RNA extraction, demonstrating a state-of-the-art performance of the system. As results obtained may differ depending on the sample type and analytical parameters (e.g., sensitivity, limit of detection, etc.), appropriate performance characteristics need to be established by the user. For diagnostic purposes, the results shall always be assessed in conjunction with the relevant application and other findings. A summary of parameters used to produce the performance data is shown in Table 6.

Table 6: ***Summary of parameters used to produce the performance data***

Parameter	SARS-CoV-2
Sample type	Swab transport media[1] spiked with SARS-CoV-2[2]
Target	Heat-inactivated SARS-CoV-2[2]
PCR assay	RIDA®GENE SARS-CoV-2 (R-Biopharm)
PCR volume eluate	5 µL
PCR volume total	25 µL
PCR instruments	Rotor-Gene® 3000

[1] *Non-chaotropic and chaotropic transport media were tested.*

[2] *SARS-CoV-2 virus particles were obtained from INSTAND e.V. (Duesseldorf, Germany). Heat inactivation was performed for 4 hours at 60 °C.*

9.2. Assessment of linearity and limit of detection (LoD)

The experimental setup to determine the linear range and the limit of detection (LoD) of the chosen parameters and the results are described in Table 7 and 8.

Table 7: ***Experimental setup to determine the linear range and LoD***

Parameter	SARS-CoV-2
Sample type	Swab transport media[1] spiked with SARS-CoV-2[2]
Sample input volume	50 µL
Elution volume	90 µL
Dilution series	11 different virus titers (5×10^1 to 1×10^6 copies/mL)
Overall data points available[2]	43[2]

[1] *Non-chaotropic and chaotropic transport media were tested.*

[2] *Based on two independent dilution series.*

Table 8: ***Results determined for linear range and LoD***

Parameter	SARS-CoV-2
Linear range	1×10^3 to 1×10^6 copies/mL
Correlation coefficient (R^2)	0.9995
LoD	13 copies/5 µL of PCR reaction

Linear range and the limit of detection are highly dependent on the PCR assay.

9.3. Intra- and inter-run precision

Standard deviations (SD) and coefficients of variations (CVs) were determined for a dilution series within the linear range using the parameters shown in Table 9.

Table 9: ***Experimental setup to determine intra- and inter-run precision***

Parameter	SARS-CoV-2
Sample type	Swab transport media spiked with SARS-CoV-2
Sample input volume	50 µL
Elution volume	90 µL
Virus titer	High: 1×10^5 / Low: 2.5×10^3
Overall data points available	144

To determine the intra-run precision, all data within one run were produced in a single experiment. The experiment was repeated three times (Table 10). To determine the inter-run precision, data were produced in three independent runs and compared with each other (Table 11).

Table 10: ***Intra-run precision***

	Run A			Run B			Run C		
Concentration (copies/mL)	**Mean (Ct)**	**SD (Ct)**	**CV (%)**	**Mean (Ct)**	**SD (Ct)**	**CV (%)**	**Mean (Ct)**	**SD (Ct)**	**CV (%)**
1×10^5	29.77	0.27	0.92	29.79	0.27	0.89	29.92	0.24	0.80
2.5×10^3	35.64	2.32	6.50	35.71	2.19	6.12	35.52	2.15	6.05

Table 11: ***Inter-run precision***

	Run A–C		
Concentration (copies/mL)	**Mean (Ct)**	**SD (Ct)**	**CV (%)**
1×10^5	29.83	0.77	0.89
2.5×10^3	35.75	2.44	6.82

9.4. Compatible transport media

Table 12 summarizes the transport media that are compatible and have been successfully tested with the EchoLUTION Viral RNA/DNA Kit.

Table 12: ***Routine swab transport media and resuspension media compatible with the EchoLUTION Viral RNA/DNA Kit***

Manufacturer	Name	Type
BioEcho Life Sciences GmbH	LyseNtact Buffer New Formula	Chaotropic
Roche®	Cobas® PCR Media (Uni Swab Sample Kit)	Chaotropic
Heinz Herenz Germany	LMS-Swab Amies	Non-chaotropic
Commercially available	TE buffer (for resuspension of stool)	Non-chaotropic
Commercially available	PBS buffer (for resuspension of dry swabs or stool)	Non-chaotropic

These data support that both chaotropic and non-chaotropic media are compatible with the EchoLUTION Viral RNA/DNA Kit. Other media not listed in the table above need to be validated before use. To use transport media not listed here, please contact us for support. Samples stored in DNA/RNA Shield™ (Zymo Research®) are not compatible with the nucleic acid extraction technology of the EchoLUTION Viral RNA/DNA Kit.

9.5. Compatible RT-qPCR assays

RT-qPCR assays that have been successfully tested in combination with the EchoLUTION Viral RNA/DNA Kit are summarized in Table 13.

Table 13: ***RT-qPCR assays tested successfully in combination with the EchoLUTION RNA/DNA Kit***

Manufacturer	PCR Assay
R-Biopharm®	RIDA®GENE SARS-CoV-2
Altona® Diagnostics	RealStar® SARS-CoV-2 RT-PCR Kit 1.0
R-Biopharm®	RIDA®GENE Viral Stool Panel III
Mikrogen® GmbH	ampliCube® Gastrointestinal Viral Panel 1

10. TROUBLESHOOTING

Observation	Comments and suggestions
Late Ct values	**Insufficient amount of viral material** If an insufficient amount of material was used e.g., because the swab sample was taken incorrectly, late Ct values might occur. **No concentration during extraction** Compared to bind-wash-elute methods the EchoLUTION technology is based on a single-step without concentration of the sample. This can lead to a Ct shift when compared to bind-wash-elute methods, but positive samples are still detected. This means that the threshold to define positive and negative results needs to be adapted based on the used extraction kit and downstream assay. To reduce this Ct shift, we have established an alternative protocol for dry swabs skipping the 1:1 dilution at the beginning of the extraction. Please see chapter 7, section 2.C (Alternative protocol to achieve higher concentration of nucleic acids: Dry nasopharyngeal swabs and dry oropharyngeal swabs).

Late Ct values (continuation)	**PCR inhibition** Another reason could be PCR inhibition (see PCR inhibition).
Late or no Ct values of Internal Control (IC) or In-Process Control (IPC)	**Small fragment size of IC/IPC** Please check your IC/IPC for the fragment size. To function as an extraction control with the EchoLUTION Viral RNA/DNA Kit, the RNA or DNA fragment used as the IC/IPC must have at least 500 nucleotides to ensure that it passes through the matrix. **Addition of control without lysis buffer** During sample preparation, be sure to add the IC/IPC to the LyseNTact Buffer New Formula before the sample is added. The addition of the IC/IPC to the sample in the absence of the lysis buffer may result in complete loss of IC/IPC in the sample due to the presence of RNases.
PCR inhibition	**Incompatible transport medium** An incompatible transport medium can lead to PCR inhibition. Please check Table 12 for validated compatible media. **Incompatible PCR assay** If the PCR assay is not listed in Table 13, there may be an incompatibility with your assay. **Columns overload (stool samples)** Check whether more than 25 mg primary stool per mL buffer have been resuspended. A higher concentration of primary stool in the resuspended sample may show up as PCR inhibition.
Occurrence of cross-contamination	**Centrifuge settings** Excessive deceleration of the centrifuge may lead to cross-contamination of the samples from one well into the other. Experience shows that this problem only occurs with deceleration times of 2–3 seconds

Occurrence of cross-contamination (continuation)	from 1000 × g to 0 × *g*. Ideally, this deceleration should take between 15–20 seconds. **Contaminated pipettes** The use of contaminated pipettes can lead to cross-contamination. We recommend a separate set of pipettes for sample preparation and PCR preparation, which should be cleaned thoroughly at regular intervals. It is also recommended to use filter tips for all pipetting steps involving samples. Furthermore, it is advised to use a separate pipette for the IC/IPC and positive control of the used PCR assays. **Handling of samples** In general, work cautiously using sterile microbiological practices when working with DNA and/or RNA. To avoid risk of contamination, always wear gloves while handling reagents and DNA and/or RNA samples. Replace gloves regularly and keep tubes closed when possible. The use of pipette tips with filters is recommended.
Non-tested transport media	If a transport medium does not appear on the list in this manual (Table 12), we might be able to test your transport medium for compatibility. Please contact us for further assistance.
DNA/RNA yield and concentration is low or **Poor performance in downstream experiments**	**Tilted matrix bed** A tilted matrix bed can lead to inappropriate sample flow through and therefore insufficient time of interaction with the matrix surface, which can lead to poor extraction performance. If you observe that the matrix is tilted and not flat, the Purification Plate was stored incorrectly. Always make sure that the Purification Plates are stored in vertical position (label facing up). **Incorrect lysis conditions** For the lysis step it is important to stick to time and temperature mentioned in the protocol, as longer lysis time can lead to degradation of the RNA and

DNA/RNA yield and concentration is low or **Poor performance in downstream experiments (continuation)**	insufficient lysis can lead to lower yields of DNA or RNA. Especially for stool samples, please use the recommended materials as other thermal shakers and accessories might not be able to reach the needed temperatures. This step is especially crucial for hard-to-lyse viruses such as rotaviruses. **Loading of purification matrix** The correct loading of the column is crucial for experimental outcome. Pipet slowly, drop-by-drop, and vertically onto the middle of the 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 not available, calculate the 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.
Eluate is missing or volume to low	**Insufficient lysate volume** Make sure that 90 µL of lysate have been loaded onto the column. **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.
Degraded RNA	**Incorrect lysis conditions** In the lysis step it is important to use time and temperature mentioned in the protocol, as longer lysis time can lead to degradation of the RNA. **Contamination with RNase** RNases digest RNA very efficiently, even small amounts can digest the RNA and lead to poor experimental outcome. Even though, included materials and solutions are RNase-free, RNases can

Degraded RNA (continuation)	be introduced while handling the samples. RNase contamination of your samples needs to be avoided. The working area and materials need to be RNase-free throughout the whole procedure, see section 2.2 for detailed instructions. We highly recommend using specific workplaces and equipment that have not been used in DNA preparations including RNase digests. Always keep extracted RNA cold.
Degraded DNA	**Incorrect lysis conditions** In the lysis step it is important to use time and temperature mentioned in the protocol, as longer lysis time can lead to degradation of the DNA.
Tilted matrix in column	**Incorrect storage of Purification Plate** If you observe that the matrix is tilted and not flat after centrifugation, the Purification Plate was stored incorrectly. Always make sure that the Purification Plates are stored in vertical position (label facing up).

For questions and further troubleshooting, please contact us!

11. LIMITATIONS OF USE

Limitations regarding EchoLUTION Viral RNA/DNA Kit are listed below:

- Strict compliance with the user manual is required for nucleic acid purification. Following good laboratory practices is crucial for the successful usage of the product. Appropriate handling of the reagents is essential to avoid contamination or impurities.
- For swab samples, only materials specified in this user manual for the detection of viral targets should be used. If a sample is not directly processed, store samples according to the manufacturer's instructions of the collection/transport tube before use.
- False-negative results may occur if a specimen is improperly collected, transported, stored, or handled. False-negative results may also occur if inadequate numbers of viral particles are present in the sample material.
- Internal Controls (IC) or In-Process Controls (IPC) for corresponding downstream assays (not included in EchoLUTION Viral RNA/DNA Kit and generally provided by the manufacturer of the downstream assay) must not be added directly to the sample and the IC/IPC should be > 500 nucleotides in length.
- The centrifuge rotor should be able to accommodate plate stacks of 5 cm of height.
- The proof of principle for the EchoLUTION Viral RNA/DNA Kit was evaluated and confirmed using state-of-the-art PCR. Performance parameters are highly dependent on the used PCR assay and system.
- There is no international standard for setting the threshold to define positive and negative results, which could influence the results as well. Appropriate performance characteristics need to be established by the user, particularly in conjunction with downstream applications other than RT-qPCR. Any result shall be interpreted within the context of all relevant clinical and laboratory findings.

12. SYMBOLS

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

Table 14: ***EchoLUTION Viral RNA/DNA Kit symbols***

Symbols	Description
	Manufacturer
	Conformité Européene/European Union conformity marking
	For in-vitro diagnostic use
	Product number
	Batch code
	Contains sufficient for < n > reactions
	Temperature limitation
	Do not re-use
	Expiration date
	Consult instructions for use

13. REVISION HISTORY

Table 15: ***Revision history***

Version	Date of issue (MM-YYYY)	Modifications
001	**09-2023**	Initial release

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!

+49 221 99 88 97-0
+1 781 497-8783

contact@bioecho.de

BioEcho Life Sciences GmbH
BioCampus Cologne, Main Building
Nattermannallee 1
50829 Köln/Cologne, Germany

BioEcho Life Sciences Inc.
400 Tradecenter Suite 6900
Woburn, MA 01801, USA

www.bioecho.com