EchoLUTION

Viral RNA/DNA Swab Kit Plus

USER MANUAL

CE IVD

REF	REF
012-051-002	012-050-002
012-051-008	012-050-008
012-051-016	012-050-016
012-102-002	012-101-002
012-102-008	012-101-008
012-102-016	012-101-016

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

BioEcho | User Manual | Version 1 - 2021

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1. INTENDED PURPOSE

The BioEcho EchoLUTION Viral RNA/DNA Swab Kit Plus is intended as an accessory for the isolation of viral RNA and DNA (depending on the viral particle to be detected) from nasopharyngeal and genital swabs as well as stool samples. The excellent purity of the isolated viral nucleic acid isolated with the EchoLUTION Viral RNA/DNA Swab Kit Plus allows immediate use in downstream applications such as PCR assays.

The EchoLUTION Viral RNA/DNA Swab Kit Plus is intended for research purposes and *in vitro* diagnostics (IVD) use in a professional laboratory environment.

2. EXPLANATION OF THE KIT

The EchoLUTION Viral RNA/DNA Swab Kit Plus is characterized by the so-called reverse chromatography principle, which reduces the extraction time with consistent sensitivity compared to state-of-the-art methods.

The EchoLUTION Viral RNA/DNA Swab Kit Plus enables, in connection with appropriate (IVD) downstream applications (e.g., RT-qPCR), fast and reliable detection of viral infections. This clinical benefit enables clinicians to consider timely clinical interventions or the exclusion of infection. Based on the clinical evidence, EchoLUTION Viral RNA/DNA Swab Kit Plus achieves the clinical benefit of:

- short processing time
- few protocol steps
- high sample throughput with minor equipment and capital invest
- generic protocol for the most common routine swab transport media and dry swabs

2.1. Purification principle

The key steps of the EchoLUTION viral nucleic acid isolation procedure are:

1. Lysis

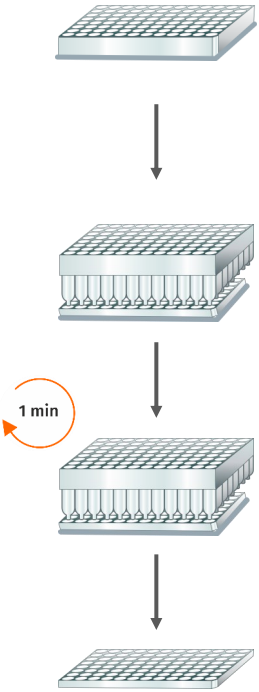
Addition of 1 Vol. LyseNtact buffer to 1 Vol. sample and resuspension on lysis plate. The LyseNtact buffer ensures immediate lysis of virus particles, inactivation of nucleases, and stabilization of nucleic acids. No additional pipetting, incubation, or heating steps are necessary.

2. Transfer of lysate

The viral lysate is loaded onto the 48/96-well purification plate filled with the purification matrix.

3. Purification

The lysate is processed by a 1-minute centrifugation step. Interfering substances, such as proteins, cell debris, and PCR inhibitors, are removed by the purification matrix. The flow-through contains the viral RNA/DNA.



The isolated viral RNA/DNA is ready-to-use and meets the quality standards required for highly sensitive quantitative downstream analysis.

3. MATERIALS

3.1. Materials provided

The kit consists of three components (Table 1): purification plate, elution plate, and LyseNtact buffer (only in the Plus Kit version). The purification plate is available in different types depending on the preparation process. Please contact BioEcho for purchase options.

Table 1: Kit contents

Kit	REF	Reactions	Purification Plate 48	Purification Plate 96	Elution Plate 96	LyseNtact Buffer
EchoLUTION Viral RNA/DNA Swab Kit Plus (2 x 48)	**012-051-002**	2 × 48	2	-	2	6 ml
EchoLUTION Viral RNA/DNA Swab Kit Plus (8 x 48)	**012-051-008**	8 × 48	8	-	8	23 ml
EchoLUTION Viral RNA/DNA Swab Kit Plus (16 x 48)	**012-051-016**	16 × 48	16	-	16	2 × 23 ml
EchoLUTION Viral RNA/DNA Swab Kit Plus (2 x 96)	**012-102-002**	2 × 96	-	2	2	11.5 ml
EchoLUTION Viral RNA/DNA Swab Kit Plus (8 x 96)	**012-102-008**	8 × 96	-	8	8	45 ml
EchoLUTION Viral RNA/DNA Swab Kit Plus (16 x 96)	**012-102-016**	16 × 96	-	16	16	2 × 45 ml
EchoLUTION Viral RNA/DNA Swab Kit (2 x 48) *	**012-050-002**	2 × 48	2	-	2	-
EchoLUTION Viral RNA/DNA Swab Kit (8 x 48) *	**012-050-008**	8 × 48	8	-	8	-
EchoLUTION Viral RNA/DNA Swab Kit (16 x 48) *	**012-050-016**	16 × 48	16	-	16	-
EchoLUTION Viral RNA/DNA Swab Kit (2 x 96) *	**012-101-002**	2 × 96	-	2	2	-
EchoLUTION Viral RNA/DNA Swab Kit (8 x 96) *	**012-101-008**	8 × 96	-	8	8	-
EchoLUTION Viral RNA/DNA Swab Kit (16 x 96) *	**012-101-016**	16 × 96	-	16	16	-

*Kits without LyseNtact buffer are CE-certified in combination with the LyseNtact buffer. Kits without LyseNtact buffer are available upon request.

3.2. Materials required but not provided

A. Plate overview

Table 2: Overview plate combinations

Purification plate	**Type 2**	**Type 3**
Conditioning plate*	Type 1	Type 2
Elution plate	Type 1	Type 2
Needed plate holder height	min. 5 cm	min. 6 cm

*Please refer to section *3.4. Related products* for product numbers.

B. Conditioning plate

The conditioning plate is necessary to remove the matrix storage buffer of the purification plate. BioEcho provides two different types of conditioning plates (Table 2) depending on the used purification plate. The conditioning plate can be re-used up to 20-times and needs to be ordered separately.

C. Lysis plate

The lysis plate necessary for mixing the LyseNtact buffer with the sample material (see section *2.1 Purification principle step 1*) is not included in the kit. BioEcho offers a suitable lysis plate for sale (product number 060-004-008). However, the user can also use their lysis plate. In this case, the lysis plate should be a 96-well plate with a capacity of at least 200 – 300 µl per well.

For stool sample lysis, 2 ml microcentrifuge tubes are needed and not provided in the kit.

3.3. Laboratory equipment needed

A. Safety cabinet

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

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

B. Plate centrifuge

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

- Standardized Society for Biomolecular Screening (SBS) format (127.76 mm × 85.48 mm × 14.35 mm).
- At least 1000 x g.
- Details on needed plate holder height is shown in Table 2.

For support on suitable centrifuges, please contact BioEcho.

C. Pipetting equipment

Pipetting can be performed using a single-channel pipette as well as a multi-channel pipette. Pipette tips with filters should always be used for pipetting infectious samples. A second pipet is needed for the positive control. Positive controls must never be pipetted with the same pipette as the samples.

3.4. Related products

BioEcho offers several products related to the EchoLUTION Viral RNA/DNA Swab Kit Plus. For a detailed description of these products, see Table 3.

Table 3: Related products to the EchoLUTION Viral RNA/DNA Swab Kit Plus

Product name	REF
LyseNtact Buffer (500)	**012-112-500**
Lysis Plate 96, Type 2	**060-004-008**
Adhesive Foil (air-permeable, 50)	**050-007-050**
EchoSAFE Viral Transport Medium (0.5 l)	**030-004-005**
EchoLUTION Viral RNA/DNA Swab Kit Plus (50) **Spin Columns - research use only**	**012-002-050**

EchoLUTION Viral RNA/DNA Swab Kit Plus (250) Spin Columns - research use only	**012-002-250**
Conditioning plate 96 Type 1 (2)	**060-001-002**
Conditioning plate 96 Type 2 (2)	**060-001-002-001**
Conditioning plate 96 Type 1 (8)	**060-001-008**
Conditioning plate 96 Type 2 (8)	**060-001-008-001**

4. STORAGE AND STABILITY

4.1. Kit and reagents

- The kit is shipped at ambient temperature.
- The purification plates and the LyseNtact buffer are stable at 2 – 8 °C until the expiration date printed on the label. Other components can be stored at room temperature (15 – 25 °C).
- Longer storage in the refrigerator or at room temperature is not recommended!

For a more detailed explanation see Table 4 and 5.

Table 4: LyseNtact lysis buffer

Function	**Virus lysing activity**
Chemical stability	Stable at 2 – 8 °C for up to 12 months (unopened)
In-use stability	Upon opening the bottle: Stable at 2 – 8 °C for 6 months
Possibility of hazardous reactions	Contact with acids liberates very toxic gas
Incompatible materials	Strong acids, strong oxidizing agents

Table 5: Purification plate 48/96-well format.

Function	**Nucleic acid purification**
Chemical stability	Stable at 2 – 8 °C for up to 18 months (unopened)
Possibility of hazardous reactions	None
Incompatible materials	None

4.2. Specimen collection

Nasopharyngeal swabs can be collected with numerous swab types, both dry and with transport media. Table 6 summarizes transport media, which are compatible and have been successfully tested with the EchoLUTION Viral RNA/DNA Swab Kit Plus.

Table 6: Routine swab transport media compatible with the EchoLUTION Viral RNA/DNA Swab Kit Plus

Manufacturer	Name	Type
BioEcho Life Sciences GmbH	EchoSAFE Viral Transport Medium	chaotropic
CoWin Biosciences	Viral Sample Preservation Solution	chaotropic
Prestige Diagnostics	Single-Use Specimen Container	chaotropic
Procomcure Biotech	Phoenix Protect DNA/RNA Conversation Solution	chaotropic
Roche	Cobas®PCR Media	chaotropic
Copan	eSwabs™	non-chaotropic
Copan	UTM	non-chaotropic
Heinz Herenz Germany	LMS-SWAB	non-chaotropic
Hologic	Specimen Lysis Tube	non-chaotropic
PBS or TRIS Buffer	in-house production	Resuspension of dry swabs

For transport media not listed here, please contact BioEcho for support.

For viral RNA and DNA extraction from stool samples, 10 – 20 mg stool can be used and is suitable for both fresh and frozen samples.

For optimal performance of the EchoLUTION Viral RNA/DNA Swab Kit Plus, sampling and transport must be performed according to the manufacturer's recommendations.

4.3. Storage and stability of purified nucleic acids

To ensure optimal performance of the purified nucleic acids, proceed immediately with PCR/RT-PCR setup or any other downstream application. If this is not possible, store RNA at -70 °C and DNA at -20 °C and prevent freeze-thaw cycles.

Handling of RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and only minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the purification procedure.

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 MSDS. Please contact BioEcho for the MSDS.

A. LyseNtact buffer

Hazard pictograms:

GHS07: Harmful

GHS05: Corrosive

GHS09: Environmental hazard

Hazard statements:

H302 + H312 + H332:	Harmful if swallowed, in contact with skin, or if inhaled.
H314:	Causes severe skin burns and eye damage.
H315:	Causes skin irritation.
H318:	Causes serious eye damage.
H400:	Very toxic to aquatic life.
H410:	Very toxic to aquatic life with long-lasting effects.
H412:	Harmful to aquatic life with long-lasting effects.

Precautionary statements:

Prevention

P273:	Avoid release to the environment.
P264:	Wash hands thoroughly after handling.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P261:	Avoid breathing dust/fume/gas/mist/vapors/spray.

Response

P301 + P330 + P331:	IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
P302 + P352:	IF ON SKIN: Wash with plenty of soap and water.
P308 + P310:	IF exposed or concerned: immediately call a POISON CENTER or doctor/ physician.
P305 + P351 + P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.
P303 + P361 + P353:	IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
P313:	Get medical advice/ attention.

Storage

P411:	Store at temperatures not exceeding 2 – 8 °C.

Disposal

P501:	Dispose of contents/containers to an approved waste disposal plant.

B. Other components

Hazard pictograms:

Not applicable.

Hazard statements:

Not applicable.

Precautionary statements:

Not applicable.

6. DISPOSAL

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

A. LyseNtact buffer

Very toxic to aquatic organisms with long-term 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 product residues. Therefore, guarantee the dispose of contents/container under local/ regional/ national/ international regulations.

B. Purification plate 48/96 and other components

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, which should be disposed of by local/ regional/ national/ international regulations.

7. PROTOCOL

The BioEcho EchoLUTION Viral RNA/DNA Swab Kit Plus is intended as an accessory for the isolation of viral RNA or DNA (depending on the viral particle to be detected) from nasopharyngeal, genital swabs, and stool samples.

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

Preparation before starting:

Set plate centrifuge to 1,000 x g.

IMPORTANT NOTE: *Choose x g (RCF), NOT RPM unless stated otherwise.*

1. Purification plate preparation

- Detach first lower and then upper foil from purification plate.
- Place purification plate on top of conditioning plate.
- Centrifuge 1 min at 1,000 x g, discard flow-through.
- Place purification plate on top of elution plate.

IMPORTANT NOTE: *Centrifuge rotor needs to hold plate sandwiches of 5 cm (Purification plate type 2) or 6 cm (Purification plate type 3) of height, as described in Table 2. Conditioning plates can be reused.*

2. Lysis plate preparation

- Add 50 µl of LyseNtact buffer to each well of the lysis plate.
- Add 0 – 20 µl of Internal Control (IC) to each well.

NOTE:

- *The IC is not provided by BioEcho. IC added before the purification step should be > 500 nucleotides in length. Please use the IC according to the manufacturer's protocol.*
- *Positive controls must never be pipetted with the same pipette as the samples.*

3. Sample preparation and viral lysis

Swabs in transport media:

- Carefully vortex swab in transport media.

- Add 50 µl of Swab media (e.g., Copan UTM, eSwab medium) to the prepared lysis plate.

NOTE:

– *With Amies agar swabs, avoid the carry-over of agar particles.*

Dry swabs:

- Rinse swab in 300 – 700 µl of EchoSAFE Viral Transport Medium, Tris buffer (e.g., 50 mM), or PBS of **pH 7.2 – 8.5** to dissolve the viral particles.
- Carefully vortex the sample.
- Add 50 µl of the resuspended sample into the prepared lysis plate.

NOTE:

– *Do not use more solution volume than needed; 300 –700 µl is usually sufficient.*

Stool samples:

- Depending on sample consistency, weigh 10 – 20 mg stool in a 2 ml microcentrifuge tube (not provided), and place tube on ice.
- Resuspend the stool sample in 600 µl Tris buffer (e.g., 50 mM) or PBS of pH 7.2 – 8.5.
- Add 50 µl of the resuspended mixture into the prepared lysis plate.

4. Viral RNA and DNA purification

- Transfer 90 µl sample mixture to 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 8-channel pipette or robot). Do not touch the matrix bed with the pipette tip during sample loading!*

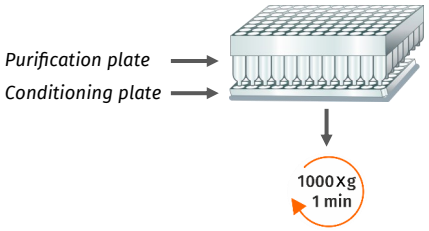
- Centrifuge loaded purification plate on top of elution plate for 1 min at 1,000 x g.
- Purified viral RNA/DNA is in the flow-through and ready-to-use.

Immediate downstream analysis is advised. In case of time-shifted downstream analysis, storing of RNA at -70 °C and DNA at -20 °C is highly recommended.

7.1. Quick protocol

1. Purification plate preparation

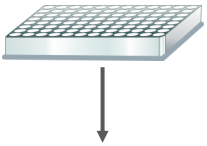
- Detach first lower and then upper foil from purification plate.
- Place purification plate on top of conditioning plate.
- Centrifuge and discard flow-through.
- Place purification plate on top of elution plate.



2. Lysis plate preparation

To each well add:

- 50 µl of LyseNtact buffer.
- 0 – 20 µL of Internal Control (IC). IC should be >500 nucleotides in length.

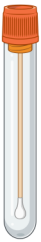


3. Sample preparation and viral lysis

Swabs in transport media

- Carefully vortex sample.
- Add 50 µl of Swab media to the lysis plate.

Dry swabs

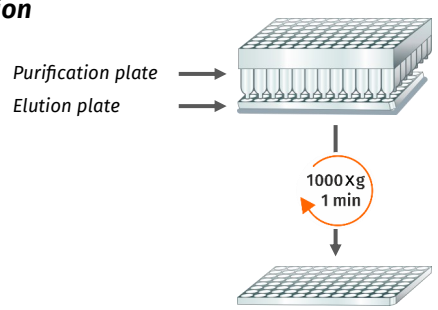


- Rinse swab in 300 – 700µl of EchoSAFE Viral Transport Medium, Tris buffer (e.g., 50 mM), or PBS of **pH 7.2 – 8.5.**
- Carefully vortex sample.
- Add 50 µl of the resuspended sample to the lysis plate.

Stool sample

- Transfer 10 – 20 mg stool sample in a 2 ml tube.
- Resuspend sample in 600 µl of Tris buffer or PBS **pH 7.2 – 8.5.**
- Take 50 µl for lysis
- Add 50 µl of the resuspended sample to the lysis plate.

4. Viral RNA and DNA purification



- Transfer 90 µl sample mixture to the purification plate.
- Purified viral RNA/DNA is in the flow-through and ready-to-use.
- Storing of RNA at -70 °C and DNA at -20 °C is highly recommended.

8. QUALITY CONTROL

In accordance with BioEcho's Quality Management System, each lot of EchoLUTION Viral RNA/DNA Swab Kit Plus is tested against predetermined specifications to ensure consistent product quality.

9. ANALYTICAL PERFORMANCE CHARACTERISTICS

9.1. Experimental setup

The EchoLUTION Viral RNA/DNA Swab Kit Plus was used according to the manufacturer's instructions for isolation and purification of total nucleic acids (RNA/DNA) from biological specimens for *in vitro* diagnostic purposes.

Representative performance data are shown below. The data show exemplarily a SARS-CoV-2 RNA extraction to demonstrate the 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 7.

Table 7: Summary of parameters used to produce the performance data shown below

Parameter	**SARS-CoV-2**
Sample type	Swab transport media[1] spiked with SARS-CoV-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. (Düsseldorf, 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 8 and 9.

Table 8: Experimental setup to determine the linear range and LoD/LoQ

Parameter	SARS-CoV-2
Sample type	Swab transport media[1] spiked with SARS-CoV-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	43[2]

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

[2]Based on two independent dilution series.

Table 9: Results determined for linear range and LoD

Parameter	SARS-CoV-2
Linear range	1×10^3 to 1×10^6 copies/ml
The correlation coefficient (R^2)	0.9995
LoD	13 copies/5 µl 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 dilution series within the linear range using the parameters shown in Table 10.

Table 10: Experimental setup to determine intra- and inter-run precision

Parameter	SARS-CoV-2
Sample type	Swab transport media[1] 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[2]

To determine the intra-run precision, all data were produced in a single run (Table 11). To determine the inter-run precision, data were produced in three independent runs (Table 12).

Table 11: Intra-run precision

	RUN A			RUN B			RUN C		
Concentration (copies/mL)	**Mean (Cq)**	**SD (Cq)**	**CV (%)**	**Mean (Cq)**	**SD (Cq)**	**CV (%)**	**Mean (Cq)**	**SD (Cq)**	**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 12: Inter-run precision

	RUN A-C		
Concentration (copies/mL)	**Mean (Cq)**	**SD (Cq)**	**CV (%)**
1×10^5	29.83	0.27	0.89
2.5×10^3	35.75	2.44	6.82

10. TROUBLESHOOTING

Comparison of the EchoLUTION technology to silica technologies – General aspects and handling

General aspects

- In the *EchoLUTION technology,* nucleic acids are not bound to a membrane or magnetic bead and can migrate freely through the filter matrix. Unwanted components of the lysate are removed from the sample and remain in the purification plate. The advantages of the EchoLUTION technology are:
  1. minor loss of nucleic acids
  2. elimination of time-consuming washing steps
  3. avoidance of 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 washed away by repeated washing with chaotropic and alcohol-containing wash buffers. Eventually, the nucleic acids are eluted with an aqueous buffer in the desired volume. Since the repeated washing steps are time-consuming, silica-based technologies are not optimal for high-throughput processing.

General handling

The EchoLUTION purification matrix is a chromatographic column. As with all chromatographic columns, the column 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 PCR inhibition. Therefore, when applying the lysate to the column **avoid touching the surface** of the filter matrix and pipet **the sample very slowly (ideally dropwise).**

Late Cq-values

- An insufficient amount of material was used.
- PCR inhibition occurred (see PCR inhibition).

Late or no Cq values of Internal Control (IC)

- Please check your IC for the recommended fragment size. A basic requirement for the functioning of an extraction control is an IC DNA/RNA fragment with a minimum size of approx. 500 nucleotides to ensure that it passes the matrix.
- During sample preparation, be sure to add the IC to the lysis buffer before adding the sample. The addition of the IC to the sample without the presence of the lysis buffer may result in complete loss of IC in the sample due to the presence of RNases.

Successfully tested RT-qPCR assays in combination with this kit

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

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

Manufacturer	PCR Assay
Anchor Diagnostics	Anchor SARS-CoV-2 PCR Kit
BAG	ViroQ SARS-CoV-2
R-Biopharm	RIDA®GENE SARS-CoV-2
Ingenetix	ViroReal® Kit SARS-CoV-2
Roche	LightMix Modular Sarbecovirus E-gene
Seegene	Allplex™ 2019-nCoV Assay
Thermo Fisher Scientific	Applied Biosystems TaqPath COVID-19
TIP Molbiol	ModularDx Kit SarbecoV E-gene EAV

PCR inhibition

- Inhibition of the RT-qPCR reaction is characterized by an increase in Cq value and a change in amplification curve morphology. However, increased Cq values also may indicate lower amounts of the target.

- If an incompatible transport medium was used, please contact BioEcho for further support.
- If there is no doubt about inhibition, the first step is to reduce the lysate volume from 90 µl to 70 µl. This will result in a larger purification capacity as more filter matrix is available per sample volume. If inhibition is still observed, the customer should contact BioEcho technical support for a possible solution.

Occurrence of cross-contamination

- Excessive deceleration of the centrifuge may lead to a cross-contamination of the samples from one well into the other. Experience shows that this problem only occurs with decelerations within 2 – 3 seconds. Ideally, the deceleration of the centrifuge should take between 15 – 20 seconds.
- The use of contaminated pipettes can lead to cross-contaminations. BioEcho recommends 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 and positive control of the used PCR assays.
- If the customer is concerned about cross-contamination by aerosols during the second centrifugation step, BioEcho offers a special air-permeable adhesive foil for the purification plate that retains aerosols and thus avoids cross-contamination. The adhesive foil is not included in the kit but can be ordered from BioEcho under the product number 050-007-050. It is generally not advisable to use your own foils, as non-air-permeable foils create a vacuum inside the plate during centrifugation, which leads to inadequate elution.

If cross-contamination still occurs, please contact BioEcho for a detailed analysis of the problem.

Lysate and eluate stability

- Lysate and eluate stability were tested with different incubation time points using IC RNA and eSwab™ transport medium with added saliva.
- LyseNtact buffer shows excellent nuclease inactivation. The RNA/DNA Lysate is stable in the lysis buffer for a sufficiently long period during the routine application.

- Storage of RNA/DNA Eluate at - 70 °C without RNA loss is possible for > 3 days.

Shelf life of purification plate after preparation

Especially with high sample numbers, many customers want to decouple the preparation of the purification plate from the actual extraction. BioEcho recommends preparing the purification plate shortly before processing the sample. However, experience shows that the functionality of the prepared purification plate is still given 24 hours after its preparation, provided that it has been stored at 2 – 8 °C.

Centrifuge speed setting

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 below, or the QR Code:

www.geneinfinity.org
/sp/sp_rotor.html

Occurrence of a white precipitate after adding the transport medium to the lysate

Some transport media (e.g., Hologic Specimen Lysis Tube) showed a white precipitate when mixed with the LyseNtact buffer. This indicates the presence of sodium dodecyl sulfate. In our experiments, no restriction or inhibition was observed with the Hologic Specimen Lysis Tube transport medium. The white precipitate is completely removed by the purification matrix and a transparent eluate was obtained. In case of further problems please contact BioEcho for assistance.

Suitable transport media

If a transport medium does not appear on the list in this manual (Table 6), BioEcho will be happy to test the transport medium for compatibility. Please contact BioEcho for further assistance.

Occurrence of liquid in the elution plate after elution although no sample was applied

After plate preparation, the matrix is not completely dried out and contains an insignificant amount of transport buffer. Since the filter matrix is still wet, second centrifugation (in this case the elution step) leads to further drainage of the matrix and residual liquid could show up in the elution plate although no sample was loaded. This is completely normal and no reason for concern.

Automation of the extraction

Some customers have already established the method on automated liquid handling systems. These include systems of Tecan, Hamilton, Perkin Elmer, or Flow Robotics. Some of the mentioned companies even offer full automation, including barcode recognition and built-in centrifugation. BioEcho will be happy to work with the system manufacturers to develop an automation solution tailored to your needs. Please contact BioEcho for further information.

11. LIMITATIONS OF USE

Limitations regarding EchoLUTION Viral RNA/DNA Swab Kit Plus are listed as follows:

- 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 contaminations or impurities.
- For swab samples, only materials specified 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.

- Samples stored in DNA/RNA Shield™ (Zymo Research) are not compatible with the nucleic acid extraction technology of the EchoLUTION Viral RNA/DNA Swab Kit Plus.
- 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) from respective downstream assays (not included in EchoLUTION Viral RNA/DNA Swab Kit Plus and generally provided by the manufacturer of the downstream assay) must not be added directly to the sample and the IC should be > 500 nucleotides in length.
- The centrifuge rotor needs to be able to hold plate sandwiches of 6 cm of height.
- The proof of principle for the EchoLUTION Viral RNA/DNA Swab Kit Plus was evaluated and confirmed using state-of-the-art PCR. Performance parameters are highly dependent on the used PCR assay and system. Also, there are only recommendations but no international standard for setting the Cq threshold, which could influence the results as well.
- Appropriate performance characteristics need to be established by the user, particularly in conjunction with any other downstream application. Any result shall be interpreted within the context of all relevant clinical and laboratory findings.
- Depending on the competitor kit used as a reference, the comparison will be different.

SYMBOLS

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

Symbol	Description
	CE marking of conformity: this device is in conformity with the applicable requirements for CE marking of an in vitro diagnostic medical device in the European Economic Area.
	In vitro diagnostic medical device
	Manufacturer
	Product number
	Batch code
	Temperature limitation
	Do not re-use
	Use by date

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For questions, further troubleshooting and also for suggestions, please contact us!

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

This user manual can be found in our shop under the respective product page.

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

+49- 221 9988 97-0

contact@bioecho.de

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www.bioecho.de

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