Instructions for Use

RealLine SARS-CoV-2 lyo
Str-Format

ASSAY KIT FOR THE QUALITATIVE DETECTION OF CORONAVIRUS SARS-COV-2 RNA BY REAL-TIME RT-PCR

In vitro Diagnostics

CE

RealLine SARS-CoV-2 lyo (Str-Format)	**VBD5580**	**96 Tests**
valid from	**October 2020**	

BIORON Diagnostics GmbH
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Explanation of symbols used in labelling

IVD	*In-vitro* Diagnostics
LOT	Batch code
REF	Catalogue number
	Contains sufficient for <n> tests
	Use-by-date
	Temperature limit
	Consult instructions for use
	Keep away from sunlight
	Manufacturer

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1. INTRODUCTION AND INTENDED USE

Clinical Information:

The RealLine SARS-CoV-2 lyo kit detects the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2, formerly 2019-nCoV). This novel virus belongs to the corona virus family and originates from China, in the city of Wuhan, Hubei province. From January 2020, it was discovered that this virus is responsible for infections that spread rapidly around the world. A few days after the first report of patients with pneumonia of unclear origin, SARS-CoV-2 was identified as the causative agent.

SARS-CoV-2 is mainly transmitted by droplet infection when coughing or sneezing and in close contact with infected persons.

The incubation period of SARS-CoV-2 can be 3 to 14 days and symptoms of SARS-CoV-2 pneumonia include fever, cough, difficulty breathing and exhaustion. In most patients, the infection manifests itself as symptoms of mild febrile illness with irregular lung infiltrations. About 3% of patients, mainly elderly and chronically ill people, may develop severe Acute Respiratory Distress Syndrome (ARDS), sometimes fatal. The disease caused by SARS-CoV-2 was named "COVID-19" by the WHO in February 2020.

RealLine SARS-CoV-2 lyo assay kit is intended for the detection of coronavirus SARS-CoV-2 RNA in clinical specimens (nasopharyngeal and oropharyngeal swabs, sputum, bronchoalveolar lavage fluid) using reverse transcription of pathogen's RNA with subsequent cDNA amplification by Real-Time Polymerase chain reaction (RT-qPCR) with fluorescence detection.

The extraction of NA from clinical specimens can be performed using the extraction kits:

RealLine Extraction 100 (REF VBC8896)
RealLine UniMag (REF VBC8883)

The results of the PCR analysis are taken into account in the complex diagnosis of diseases.

When using NA extraction kits of other manufacturers, it is necessary to use RealLine Internal Control sample (IC, VBC8881). Please note that full and valid result interpretation is not possible without IC.

The **Str-Format Kit** contains 96 tubes (0.2 ml) with lyophilized Mastermix. 50 µl of extracted DNA have to be pipetted into the tube and the ready mastermix is diluted. The kit contains reagents required for 96 tests, including control samples and the positive control sample.

The kit is intended for use with block-type cyclers: CFX™96 (Bio-Rad, USA), RealLine Cyclers (BIORON Diagnostics GmbH) and DT-96 (DNA-Technology, Russia).

Target genes:

FAM	ROX
Internal Control (RNA-IC)	**SARS-CoV-2:** N-Gene RdRp gene

The use of:

- **Extraction Kits for nucleic acids from clinical specimen from other supplier**
- **other real-time PCR devices**
- **appropriate reaction volumes, other than 50 µl**

has to be validated in the lab by the user. The special notes regarding the internal control IC have to be strongly followed.

2. KIT CONTENTS

Ready Master Mix (RMM), lyophilized	96 test-tubes
Positive Control Sample (PC)	1 tube, 1 ml
The kit is additionally supplied with optical-quality PCR-film	

3. PRINCIPLE OF THE METHOD

The detection is based on reverse transcription of a selected region of RNA of the SARS-CoV-2 virus combined with amplification of the selected cDNA fragment (consisting of repeated cycles: thermal denaturation, hybridization of primers with complementary sequences, extension of polynucleotide sequences from these primers with Taq DNA polymerase) is recorded in real-time mode.

Real-time PCR is based on the detection of the fluorescence produced by a reporter molecule, which increases as the reaction proceeds. Reporter molecule is a dual-labelled DNA-probe that specifically binds to the target region of pathogens DNA. Fluorescence signal increases due to the separation of fluorescence dye and quencher by Taq DNA-polymerase exonuclease activity during amplification. PCR consists of repeated cycles: temperature denaturation of DNA, primer annealing and complementary chain synthesis.

Threshold cycle value (Ct) is a cycle number at which the fluorescence generated within a reaction crosses the threshold and the fluorescence signal rises significantly above the background. Increased signal is due to the use of a DNA hybridization probe that is specific for the given cDNA sequence. The detected fluorescence intensity depends on initial quantity of pathogen's cDNA template in the sample.

Internal Control sample (IC) is used to monitor the possibility of false negative results (due to RNA/cDNA loss during extraction procedure or inhibitory effects of sample components). IC has to be added to each specimen and control sample prior to the NA extraction procedure.

4. SPECIFICATIONS

4.1. Analytical specificity

The **RealLine SARS-CoV-2 lyo** assay kit is ensured by the specific primers and probe. The primers and probe were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

Additionally, cross-reactivity was evaluated using the control panel containing NA of different viruses and bacteria. No false-positive results were obtained.

Viruses	
Influenza A and B viruses	Coronaviruses:
Human parainfluenza viruses	HCoV-NL63
Human adenoviruses	HCoV-OC43,
Human respiratory syncytial virus	HCoV-229E
Human metapneumovirus	HCoV-HKU1
Rhinoviruses	
Bacteria	
Streptococcus pneumoniae	*Haemophilus influenzae*
Streptococcus pyogenes	*Staphylococcus aureus*.

4.2. Analytical sensitivity of SARS-CoV-2 RNA detection was determined using probity analysis and confirmed on the following types of clinical specimens: nasopharyngeal and oropharyngeal swabs, sputum, bronchoalveolar lavage fluid.

4.3. Analytical sensitivity of **RealLine SARS-CoV-2 lyo** assay kit equals 25 copies in the test sample, which corresponds to 1×10^3 copies/ml if the extraction kits **RealLine Extraction 100** or **RealLine UniMag** are used.

Note: Analytical sensitivity depends on the sample volume, elution volume, NA extraction method, and other factors.

4.4. Diagnostic evaluation

Diagnostic sensitivity of SARS-CoV-2 RNA detection:

Clinical tests performed on 100 positive samples (nasopharyngeal and oropharyngeal swabs, sputum, bronchoalveolar lavage fluid) showed 100 % sensitivity (interval 97.0 % – 100 %, with a confidence level of 90 %).

Diagnostic specificity of SARS-CoV-2 RNA detection:

Clinical tests performed on 100 negative samples (nasopharyngeal and oropharyngeal swabs, sputum, bronchoalveolar lavage fluid) showed 100 % specificity (interval 97.0 % – 100 %, with a confidence level of 90 %).

Reproducibility of the results:

Clinical tests performed on 100 positive and 100 negative samples using two lots of the kit showed 100 % between-lot reproducibility. The tests performed on the model sample containing SARS-CoV-2 RNA in the concentration of 1×10^3 copies/ml in 10 repeats by two operators using two lots of the kit showed 100 % within-lot, within-laboratory, and between-lot reproducibility.

5. LIMITATIONS

- This assay must not be used on the clinical specimen directly. Appropriate nucleic acids extraction methods have to be conducted prior to using this assay.
- The presence of PCR inhibitors (e.g. heparin) may cause false negative or invalid results.
- When monitoring a patient the same extraction method must be used in all determinations. Otherwise, results may not be relative.
- The kit is designed for use in patients with a clinical history and/or symptoms consistent with SARS-CoV-2 infection. The kit may be used for screening purposes.
- Diagnostic sensitivity of the kit may vary depending on the pathogen prevalence and characteristics of the enrolled cohort.
- Reliable results depend on adequate specimen sampling.
- Positive results indicate active or asymptomatic infection; results should be interpreted with consideration of clinical and laboratory findings.
- Negative results indicate lack of detectable DNA but do not exclude the infection or disease.
- Potential mutations within the target regions of the SARS-CoV-2 genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- The kit is not intended to replace culture and other methods for diagnosis of urogenital infection.

6. WARNING AND PRECAUTIONS

- The kits must be used by skilled personnel only.
- To obtain reliable results, strictly follow this Instruction Manual provided with the kit.
- When handling the kit, follow the national safety requirements for working with pathogens.
- To prevent contamination, the stages of RNA isolation and PCR test run must be spatially separated.
- Avoid microbial and ribonuclease contamination of reagents when removing aliquots from reagent vials.
- Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents.
- Every workplace must be provided with its own set of variable-volume pipettes, necessary auxiliary materials and equipment. It is prohibited to relocate them to other workplaces.
- To conduct amplification reaction with real-time PCR products detection, use only disposable tips with filters.
- Never use the same tips for different samples.
- Do not pool reagents from different lots or from different vials of the same lot.
- Dispose unused reagents and waste in accordance with country, federal, state and local regulations.
- Do not use the kit after the total expiration date at the side label of the box.

7. ADDITIONAL MATERIALS AND DEVICES REQUIRED BUT NOT SUPPLIED

- Real time PCR system, like described in p.1
- RNA-Extraction Kit: **RealLine Extraction 100 or RealLine UniMag**
- Internal Control reagent (VBC8881), if the kit is used with the extraction kits of other supplier.
- Laminar safety box;
- Refrigerator;
- Half-automatic variable-volume single-channel pipettes;
- Disposable medical non-sterile powder-free gloves;
- Disposable pipette tips with aerosol barrier;
- Racks for 2.0 ml and 0.2 ml tubes
- Biohazard waste container;
- Razor or scalpel

8. PREPARATION OF SAMPLES

*Each group of samples undergoing the procedure of DNA isolation must include a **Positive Control sample (PC)** from this kit and a **Negative Control sample (NC)** which is a component of the DNA extraction kit.*

The assay is performed on extracted NA samples obtained from the clinical material using one of the NA extraction kits listed on p. 1, according to the Instruction Manual for the kit. If an extraction kit with magnetic particles is used, keep the tubes with extracted NA in a magnetic rack.

Specimens containing mucus (sputum, bronchoalveolar lavage fluid) should be pretreated before the NA extraction as follows. Add an equal volume of 95 % ethanol to the specimen. Mix thoroughly and keep at (18 – 26) °C for 10 min. Mix thoroughly one more time. Use 100 µl of the obtained mixture for the NA extraction. Avoid mucus clumps!

***Attention!** Storage of extracted RNA is not recommended! Perform RNA extraction and RT-PCR on the same day.*

Implementation of the Internal Control IC, the Negative Control NC and Positive Control PC samples to the extraction procedure is necessary.

When using kits of another supplier for the extraction of nucleic acids as recommended in chapter 1: add **20 µl** of **IC (VBC8881)** to each tube.

- For the NC use **100 µl** of the Negative Control Sample
- For the PC use **70 µl** of Negative Control Sample and **30 µl** of Positive Control to the tube marked PC.

After the initial opening of the tube, store PC at (2–8) °C for no more than 1 month or in 50 µl aliquots at minus (18–24) °C for up to 3 months.

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9. PROCEDURE

9.1. Preparation of the kit components

Prior to the analysis, take the kit out of the refrigerator and keep the **Ready Master Mix for PCR (RMM)** closed in the package at (18 – 26) °C for at least 30 minutes. Open the package and cut off the necessary number of tubes with RMM (including the test and control samples: 1 NC and 1 PC) with the razor or scalpel. Cut the tubes together with the covering film.

Attention: Put the remaining strips immediately back into the foil pouch, squeeze the air out and tightly close with the clip.

After initial opening of the package, store RMM at (2 – 8) °C for no more than 3 months.

9.2. **Label** the tubes with RMM for each test and control sample.

Attention! *Labels should be placed on the lateral side of the tubes, leave optical film clean.*

9.3. Add **50 µl** of corresponding isolated RNA solution to each tube using a separate pipette tip with filter. Tightly close the tubes with caps or seal with the PCR transparent film.

9.4. Place the tubes into the real-time PCR system.

9.5. Program real time PCR system as follows:

Step 1:	45°C	30 min	
Step 2:	94°C	1 min	
Step 3:	94°C	10 sec	50 cycles
	60°C*	20 sec	
* Measure the fluorescence at 60°C			

9.6. Select the amplification detection channels:

- detect the amplification signal of **IC cDNA** in the **FAM** channel
- detect the amplification signal of **SARS-CoV-2 viruses cDNA** in the **ROX** channel.

9.7. Program the positions of test tubes with samples, positive and negative controls according to the instruction manual for the real time PCR system in use.

9.8. Run the program.

10. DATA ANALYSIS AND INTERPRETATION

10.1 For **Positive Control** the program should detect:

- Increase of the IC DNA amplification signal in the channel **FAM** and determine the threshold cycle, **IC Ct**;
- Increase of the **SARS-CoV-2 cDNA** amplification signal in the channel **ROX** and determine the **PC Ct** value.

10.2 For **NC** the program should detect the increase of the amplification signal of IC cDNA (channel **FAM**) and determine **IC Ct**. No significant **ROX** fluorescence increase should appear.

If **Ct** value for NC in **ROX** channel **is less than or equal to 40,** this indicates the presence of contamination (see paragraph 10.7).

10.3 For each test sample the program should detect the increase of the amplification signal of IC DNA (channel **FAM**) and determine IC **Ct**.

10.4 Calculate (IC **Ct**)$_{av}$ as an average IC **Ct** of all analyzed samples (including PC and NC). IC **Ct** values that differ by more than 2 from the (IC **Ct**)$_{av}$ should be ignored. Recalculate the (IC **Ct**)$_{av}$ for the remaining values after the screening.

10.5 The sample is considered **positive**, i.e. contains **SARS-CoV-2 RNA**, if **Ct** value via **ROX** channel for this sample is less than or equals to 40.

If there is a signal in **ROX** channel above Ct 40, additional tests are recommended (analysis after new sampling or analysis using another assay kit).

10.6 The sample is considered negative, if the Ct in the **ROX** channel is not determined. If **IC Ct** value for such sample differs from (IC Ct)av value by more than 2, the result is considered equivocal. A repeated analysis of the sample, starting from the NA extraction step is required

10.7 If the **Ct** value for NC in the **ROX** channel is **determined**, it indicates the presence of contamination. In this case, all positive results of this individual PCR test run are considered **equivocal**. Actions are required to identify and eliminate the source of contamination. Repeat the analysis of all samples of this run that were determined positive. Samples that showed negative results in this run should be considered **negative**.

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11. STORAGE AND TRANSPORTATION

- Store the assay kit at (2 - 8) °C in the manufacturer's packing for the entire shelf life.
- Transport at (2 – 8) °C; transportation for up to 25 °C for no more than 10 days is allowed.
- Do not freeze the kit!
- Do not pool reagents from different lots or from different vials of the same lot.
- Strictly follow the Instruction manual for reliable results.
- Do not use kits with damaged inner packages and get in contact with BIORON Diagnostics GmbH.

- **Storage and shelf life of solutions and components of the kit after initial opening:**
  Positive Control and Negative Control sample: 1 month at (2 - 8) °C *or in 50 µl aliquots at minus (18 - 60) °C for up to 3 months*.
  Ready Master Mix (RMM): 3 months at (2 – 8) °C

ANNEX I: Settings for RealLine Cycler and DT96:

for these cyclers the measurement exposure must be adjusted. Choose the **Operation with the device** mode in the **Settings** menu, select the item **Measurement exposition:**

- **FAM** to **250**
- **HEX** and **ROX** to **1000**

Confirm that the current exposure value is saved by pressing **YES**

Attention! The specified exposure values are applicable only for RealLine Kits (REF VBDxxxx) and, if necessary, must be changed for other purposes.

Technical Support: techsupport@bioron.de

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