AzureSeq CE qPCR KIT SARS-COV-2 for 200 Reactions, AzureSeq CE qPCR KIT SARS-COV-2 for 400 Reactions INSTRUCTIONS FOR USE

For In Vitro Diagnostic Use

PROTOCOL VERSION V1.0
DOCUMENT REVISION 04
2/23/2022

CE

Table of Contents

ABBREVIATIONS 4
1. INTENDED USE 4
2. PRINCIPLE OF THE METHOD 4
3. KIT CONTENT 5
4. SHIPPING AND STORAGE 6
5. SPECIMEN COLLECTION, TRANSFER AND STORAGE 6
6. MATERIALS AND EQUIPMENT NEEDED, BUT NOT PROVIDED 9
7. WARNING AND PRECAUTION 10
8. PROCEDURE 11
9. RESULT INTERPRETATION 16
10. PROCEDURE LIMITATION 18
11. TROUBLESHOOTING 19
12. QUALITY CONTROL 20
13. PERFORMANCE CHARACTERISTICS 20
14. REFERENCES 28
15. SYMBOLS USED ON LABELS 29
16. CONTACT INFORMATION 29

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

Revision history

Revision	Date	Summary of changes
1	01-Oct-2020	First version
2	22-Oct-2020	Sample collection, handling and storage (section 5), qPCR setup (section 8.2) and references (section 14) have been added. Typos have been corrected.
3	09-Feb-2022	Change of Manufacturer address
4	18-Feb-2022	CE logo updated, Revision history table simplified, Kit content section extended with kit configuration for 400 samples, text revisions to match the protocol with both product configurations, recommendations on repeated use of the kit added to Section 4, Section 10 extended with the warning for false negative results in case of direct method

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

Abbreviations

In this document we refer to AzureSeq CE qPCR KIT SARS-COV-2 for 200 Reactions and AzureSeq CE qPCR KIT SARS-COV-2 for 400 Reactions products together as AzureSeq CE qPCR KIT SARS-COV-2 or in a short form as AzureSeq CE.

1. Intended Use

The AzureSeq CE qPCR KIT SARS-COV-2 is an RT-qPCR test intended for the qualitative detection of nucleic acid from the 2019-nCoV in nasopharyngeal (NP) and oropharyngeal (OP) swabs from individuals with signs and symptoms of infection who are suspected of COVID-19.
Results are for the identification of 2019-nCoV RNA. The 2019-nCoV RNA is generally detectable in nasopharyngeal and oropharyngeal swabs during the acute phase of infection. Positive results are indicative of active infection.
Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.
The AzureSeq CE qPCR KIT SARS-COV-2 is intended for use by qualified, trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

2. Principle of the method

The test is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test which is designed to detect RNA from two different regions of the SARS-CoV-2 nucleocapsid gene in nasopharyngeal (NP) and oropharyngeal (OP) swabs from patients with signs and symptoms of infection who are suspected of COVID-19. The kit primer and probe set also detects human RNase P (RP) in a clinical sample as internal control.
The target nucleic acid sequences from the genome of SARS-CoV-2 are the same sequences utilized by the CDC for their 2019 Novel Coronavirus (2019-nCoV) Real- Time RT-PCR Diagnostic Panel. These sequences are contained within 2 different regions (N1 and N2) of the virus nucleocapsid (N) gene.
Nucleic acids are isolated and purified from nasopharyngeal and oropharyngeal swabs using well established nucleic acid extraction systems. Sample input and elution volumes are system dependent. Samples collected in specific Viral Transport Medium which have undergone a heat incubation can be also used as inputs to the subsequent steps.
The purified nucleic acid, or the heat extracted sample is reverse transcribed into cDNA by combining nucleic acid with the AzureSeq CE qPCR KIT SARS-COV-2 (further referred as AzureSeq CE) master mix which is then subsequently amplified in the real time PCR instrument. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5’ nuclease activity of Taq polymerase degrades the probe,

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the real time PCR instrument.

3. Kit content

AzureSeq CE qPCR KIT SARS-COV-2 for 200 Reactions

Product code: AzureSeq-200 CE

Product Code	Product Name	Number of tubes	Volume/Tube (µL)
OA-ITMP-MM-100	2X InhibiTaq Multiplex HotStart MasterMix	2	1000
OA-RT-200	RTScript Reverse Transcriptase, 200U/uL	1	100
OA-CPPM-100uL	CoVi Primer/Probe Mix 3	2	100
OA-NFW-350uL	Nuclease Free Water	2	350

AzureSeq CE qPCR KIT SARS-COV-2 for 400 Reactions

Product code: AzureSeq-400 CE

Product Code	Product Name	Number of tubes	Volume/Tube (µL)
OA-ITMP-MM-100	2X InhibiTaq Multiplex HotStart MasterMix	4	1000
OA-RT-200	RTScript Reverse Transcriptase, 200U/uL	2	100
OA-CPPM-100uL	CoVi Primer/Probe Mix 3	4	100
OA-NFW-350uL	Nuclease Free Water	4	350

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

Note: The positive (Cat# OA-CVPC-150) and negative (Cat# OA-CVNC-150) controls are not included in the kit and are supplied separately.

4. Shipping and Storage

- The AzureSeq CE kit is shipped on dry ice. The kit components should arrive frozen. Please contact azureseq.support@omixon.com if any components are not frozen upon receipt or are compromised during shipment.
- To prevent the reagents from degradation, all components should be stored immediately at -20°C.
- It is recommended to have a back-up generator for your freezer as well as a temperature data log to ensure that the AzureSeq CE kit components remain frozen at -20°C if you work in an area prone to power outages.
- Expires 12 months after date of manufacture. Do not use it after the expiration date.
- Do not use the kit if it is defective.
- Repeated freezing/thawing must be avoided because it can cause reagent degradation that leads to decrease in sensitivity.
- Dispose of unused reagents and waste according to country regulations.

5. Specimen collection, transfer and storage

Inadequate or inappropriate specimen collection, transfer and storage can increase the probability of false negative results.

5.1 Collection

Refer to CDC's webpage for Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from Patients Under Investigation (OUIs) for 2019 Novel Coronavirus (2019-nCoV). https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html

Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing.

Follow specimen collection devices manufacturer instructions for proper collection methods.

Oropharyngeal swab

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

Use a sterile swab to wipe the posterior pharynx, avoiding the tongue. Place swabs immediately into labeled sterile tubes containing viral transport medium. Break each applicator sticks off at the score line (flocked swabs) or near the tip or cut with sterile scissors to permit tightening of the cap. Ship sample immediately on cold packs.

Nasopharyngeal swab

Insert a sterile swab into nostril parallel to the palate. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it. Place swabs immediately into labeled sterile tubes containing viral transport medium. Break each applicator sticks off at the score line (flocked swabs) or near the tip or cut with sterile scissors to permit tightening of the cap. Ship sample immediately on cold packs.

5.2 Transport

All specimen must be transported with ice cool / ice-gel box / dry ice and securely sealed and handled.
Transport of clinical samples must obey local regulations. The biosafety local regulations for SARS-CoV-2 must be followed.

5.3 Handling

During handling potentially infectious specimens, laboratory workers should wear appropriate personal protective equipment (PPE), which includes disposable gloves, laboratory coat/gown, and eye protection.
For specific instructions on the handling of clinical specimens for coronavirus disease 2019, see also the CDC's webpage mentioned above.

5.4 Storage

- Specimens can be stored at 2-8°C for up to 48 hours after collection. For storage longer than 2 days, specimens should be frozen at -70°C.
- If the sample is stored for more than 48 hours, extraction of the RNA using validated RNA isolation system is required.
- If the sample is not frozen and stored for less than 48 hours, direct approach of workflow can be used.
- Repeated freezing and thawing of a specimen should be avoided. If a specimen is kept for retesting, it should be aliquoted in different tubes to avoid freezing and thawing cycles.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

- Depending on the type of sample and the transport medium used, specific storage and, for RNA isolation, pre-treatment of the sample may be required. Please observe the instructions provided by the manufacturer.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

6. Materials and Equipment needed, but not provided

6.1. Equipment

- Real-time PCR thermal cycler able to detect FAM, HEX or ROX (or equivalent) channels
- Thermoblock (suitable for 1.5 mL microcentrifuge tubes, capable of heating up of 95°C)
- 100 µL and 1000 µL micropipettes
- 10 µL and 100 µL multichannel pipette
- Vortex mixer
- Centrifuge

6.2. Reagents

- Viral RNA/total RNA extraction kit
- Viral Transport Medium (Clinichem, Copan, Puritan, CDC VTM, mwe Medical Wire)
- CoVi Negative Control (Cat# OA-CVNC-150)
- CoVi Positive Control (Cat# OA-CVPC-150)

6.3. Materials

- Optical 96-well plates or 0.2 ml optical tubes
- Optical seal compatible with the qPCR instrument
- Disposable DNase/RNase free pipette tips with filters (10 µL, 20 µL, and 200 µL)
- DNase/RNase free 1.5 ml tubes
- Disposable powder-free gloves
- Surface decontaminant products such as "RNAse away"
- Material necessary for nucleic acid extraction

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

7. Warning and Precaution

Good laboratory practices are essential to the proper performance of this assay. Due to the high sensitivity of the test, care should be taken to handling samples and materials while performing the assay to keep reagents and amplification mixtures free of contamination.

Users should pay attention to the following:

- Read the Instructions for Use carefully before processing samples. Any deviation from procedures written here may affect optimal performance.
- Use disinfectant to clean and disinfect the area around the sample
- Decontaminate and dispose of all specimens, reagents, and other potentially contaminated materials in accordance with local regulations
- Use universal precautions when performing the assay. Handle samples as if capable of transmitting infection.
- Wear personal protective equipment throughout the assay procedure.
- Thoroughly wash hands after removing gloves, and dispose of gloves as hazardous wastes
- Do not reconstitute or dilute the reagents in volumes other than described in this IFU. Do not use less volume of the reagents other than specified in this IFU. These activities can lead to performance errors.
- Omixon cannot provide support for any problems resulting from not following the protocol steps described in this IFU.
- Do not use the product in case of detectable damage to the components (broken vials, plate, loose caps etc.).
- Do not use reagents past their expiration date.
- Do not substitute or mix the AzureSeq CE kit reagents with reagents from other manufacturers.
- All instruments must be maintained and operated according to manufacturer's instructions.
- Every workplace must be equipped with its own set of variable-volume pipettes, necessary auxiliary materials, and equipment.
- Do not pool reagents from different lots or from different vials of the same lot.
- Do not smoke, drink, eat or apply cosmetics in areas in which specimens or Kit components are handled.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

8. Procedure

The AzureSeq CE kit contains a control assay targeting the RNaseP. This is an internal control needed to confirm the presence of nucleic acid in every sample run with the AzureSeq test kit and is used to generally confirm functionality of the test kit components.

Other than that, there are no external controls provided with the AzureSeq CE test kit. Users are expected to supply commercially available positive and negative controls from Omixon as a separate product (catalog numbers are listed in Section 3) or they can use their in-house ones.

8.1. Instructions for setting up the reactions

In case of direct approach without separate RNA extraction (heat treatment), please follow the instructions from step 1. In case of starting with RNA purified with regular, validated RNA extraction kits, skip steps 1-4 and start with step 5.

8.1.1. Instructions

Recommended for use only with OP or NP swabs in Viral Transport Media (VTM) from the following manufacturers: Clinichem, Copan, Puritan, CDC VTM, mwe Medical Wire

Sample in VTM can be stored for up to 48 hours on 4°C.

1. Obtain swabbed OP/NP material in VTM.
2. Transfer 100-200µL of swabbed OP/NP VTM material into compatible DNase/RNase free tubes.
3. Heat sample for 5 minutes at 95°C.
4. Following incubation, spin the heated sample on ~1500 x rpm for 30 seconds to collect material at the bottom of the tube. Store on ice until needed. The sample is now ready to be added to the RT-qPCR reaction (see following steps).
5. Completely thaw the CoVi Primer/Probe mix 3 (brown tube/cap) by setting it on ice for ~30 minutes. Once thawed, briefly centrifuge to collect at the bottom of the tube, then add **384 µL** of nuclease free water to the tube. Mark the tube with water added.
6. Vortex the tube at max speed for 10 seconds to mix, then spin down briefly to collect at bottom of the tube.
7. Mix 2x InhibiTaq Multiplex qPCR MasterMix thoroughly (vortex at max speed, spin on 3200 x rpm for 2-4 seconds) and visually inspect that no pellet is present.
8. Proceed to master mix setup as shown below in a clean room or designated setup area.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

Reaction set-up for 20µL reaction volume:

Component	Volume/ reaction (µL)	Volume/100 reactions (µL)	Final concentration
2x InhibiTaq Multiplex qPCR MasterMix	**10**	**1000**	**1x**
RTScript Reverse Transcriptase, 200U/µL	**0.5**	**50**	**5U/µL**
Diluted Primer/Probe Mix (step #5)	**4.5**	**450**	**1x**

9. Mix the master mix by pipetting up and down repeatedly with pipette set to volume of 2X master mix added, or by capping the tube, vortexing briefly, and spinning down briefly to collect mix.
10. Distribute **15 µl** of the master mix using an appropriate pipette to all wells of a plate that will be used.
11. Add **5 µl** of the samples, positive control, or negative control to appropriate wells.
12. Seal the plate, vortex briefly or flick to mix; spin down in a centrifuge to collect the mixed samples.
13. Place the plate into the designated real-time machine and run the following program.

Recommended cycling conditions:

Cycling step	Stage	No. of cycles	Temperature (°C)	Time
RT incubation	**1**	**1**	**50**	**15 minutes**
Enzyme activation	**2**	**1**	**95**	**2 minutes**
Amplification	**3**	**45**	**95**	**3 seconds**
			60**	**30 seconds**

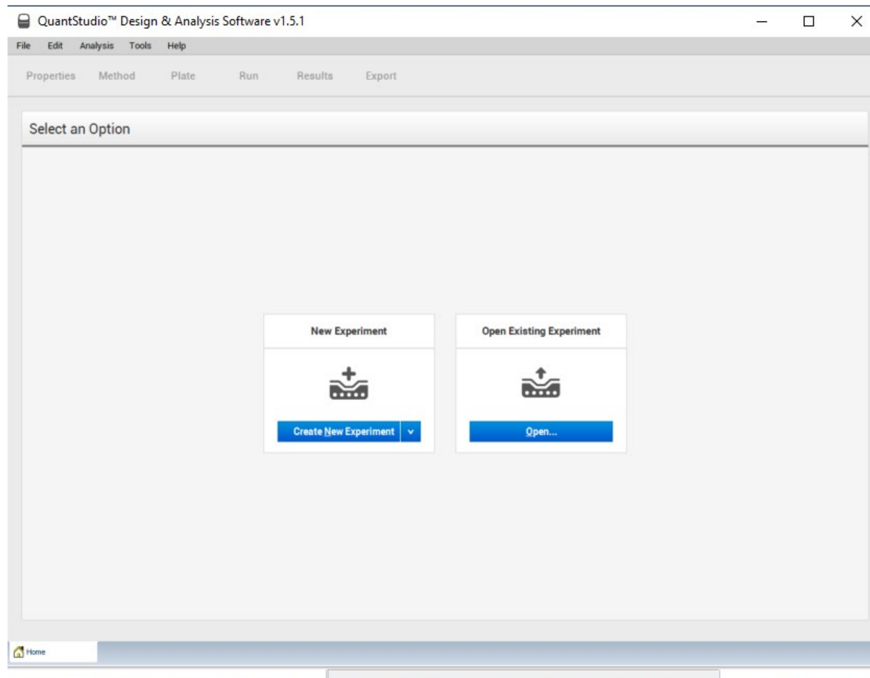
**Collect fluorescence during annealing/extension phase (60°C) step on FAM, HEX, and ROX channels (or equivalent channels).

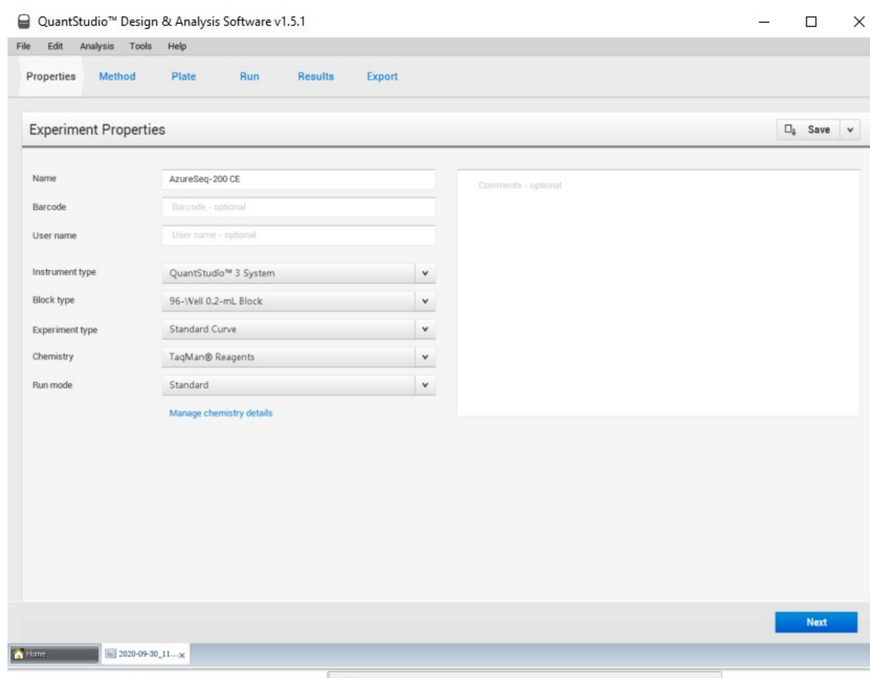
Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

8.2 qPCR setup

8.2.1 QuantStudio 3, 5, 7 Pro

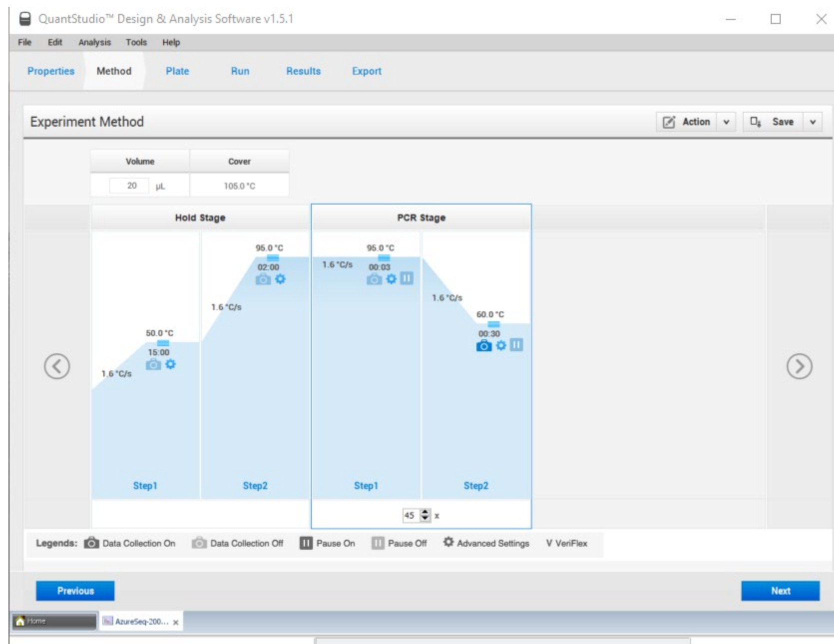
1 – Create a new experiment and then setup the Properties (Instrument Type, Block Type, Experiment Type, Chemistry, Run mode) according to the picture below.



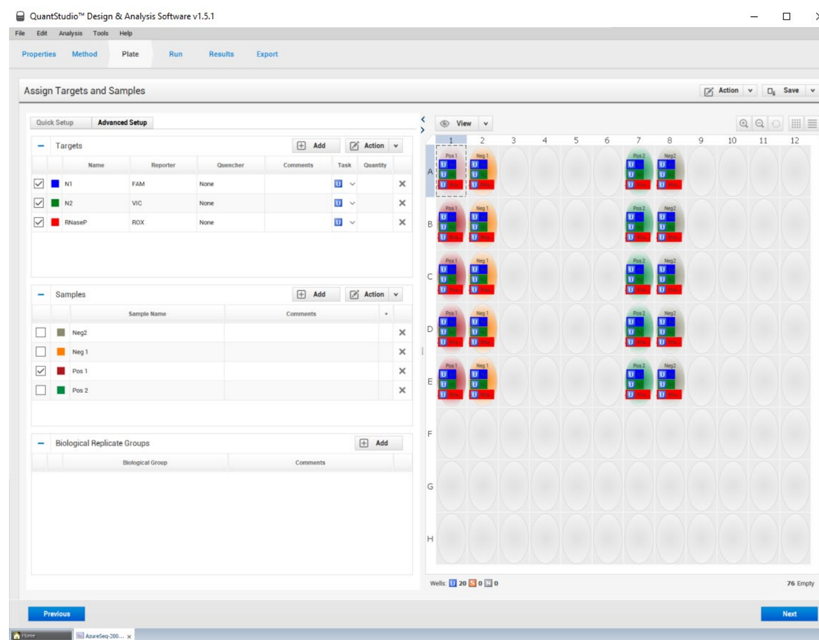


Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

2 – Set the method according to the PCR program detailed above.



3 – Set the targets and the samples on the Plate tab and assign the channels.



4 – Export the protocol to an USD drive.

5 – Load the Experiment from the USB drive on the qPCR instrument, insert the plate and start the Run.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

8.2.2 Roche LightCycler 480 System

Please refer to the LightCycler 480 instrument Operator's manual for additional information on using the instrument.

- Create a 'Detection Format' to setup the targets (N1, N2, RNase P) and channels (FAM, HEX, ROX – or equivalent).
- Restart a software to access the newly created Detection Format.
- Select the color of the plate and create a New Experiment.
- Choose the new Detection Format from the drop-down list.
- Set the Block Size to 96 and the Reaction Volume to 20 µL.
- Input the PCR program detailed in step 8.1.1.
- Set the Analysis Mode 'None' for RT incubation and Enzyme activation and 'Quantification' for the Amplification step.
- Set the Ramp to 4.4 for Stage 1, 2 and the first step of Stage 3 and to 2.2 for the second step of Stage 3.
- Load the plate and start the run.

8.2.3 BioRAD CFX96 Touch

Please refer to the CFX96™ Touch Instruction Manual for additional information on using the instrument.

- Run the CFX Manager software on the computer connected to the CFX96.
- Create a New Protocol by selecting th 'User-defined' run type.
- Click 'Edit Selected' on 'Protocol' tab to make changes to the protocol. Set the parameters of the PCR program detailed in Section 8.1.1.
- Click 'Edit Selected' on 'Plate' tab to setup the Plate.
- Click on 'Select Fluorophores', check the cyberbox of the fluorophores (FAM, HEX, ROX – or equivalent).
- Specify the positive control well, set the sampe type to "Positive Control", and set the detection fluorescences (N1 target - FAM, N2 target - HEX, Rnase P - ROX).
- Specify the negative control well, set the sampe type to "Negative Control", and set the detection fluorescences (N1 target - FAM, N2 target - HEX, Rnase P - ROX).
- Wells with clinical specimens should be specified as Unknown, set the detection fluorescences (N1 target - FAM, N2 target - HEX, Rnase P - ROX).
- In the Setting menu set the plate type to BR white.
- Go to Start Run, select Block Name (PCR instrument) to use, close Lid and start the run.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

9. Result interpretation

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. Collect fluorescence during annealing/extension phase (60°C) step on FAM (N1 marker), HEX (N2 marker), and ROX (RNaseP marker) channels (or equivalent channels). Ct values <40 are interpreted as positive by the user for the BioRad CFX96, Roche Lightcycler 480 System and QuantStudio 3, 5, and 7 Pro.

SARS-CoV-2 N1 (FAM)	SARS-CoV-2 N2 (HEX)	RNase P (Texas Red)	Result Interpretation	Action
+	+	+/-	**Positive SARS-CoV-2**	**Report results to the healthcare provider and appropriate public health agencies.**
If only one of the two targets is positive		+/-	**Inconclusive**	**Repeat test.**
-	-	+	**SARC-CoV-2 Not Detected**	**Report results to the healthcare provider.**
-	-	-	**Invalid**	**Repeat test. If still invalid, collect another specimen. If another specimen is unavailable, report to the healthcare provider.**

Use the table above as a general guide for result interpretation.
To report a patient test result, all controls within the same run must be valid and produce the expected results. If either or both of the controls is deemed invalid or produces unexpected results, no patient results from that run should be reported.

RNase P (Extraction Control)

All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 40.00 cycles (< 40.00 Ct), thus indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate:

- Improper extraction of nucleic acid/improper heat extraction from clinical materials resulting in loss of RNA and/or RNA degradation.
- Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.
- Improper assay set up and execution.
- Reagent or equipment malfunction.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:

- If the 2019-nCoV N1 and N2 are positive even in the absence of a positive RP, the result should be considered valid. It is possible that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of 2019-nCoV virus RNA in a clinical specimen.
- If all 2019-nCoV markers AND RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

2019-nCoV Markers (N1 and N2)

• When all controls exhibit the expected performance, a specimen is considered negative if all 2019-nCoV marker (N1, N2) cycle threshold growth curves DO NOT cross the threshold line within 40.00 cycles (< 40.00 Ct) AND the RNase P growth curve DOES cross the threshold line within 40.00 cycles (< 40.00 Ct).

• When all controls exhibit the expected performance, a specimen is considered positive for 2019-nCoV if all 2019-nCoV marker (N1, N2) cycle threshold growth curves cross the threshold line within 40.00 cycles (< 40.00 Ct). The RNase P may or may not be positive as described above, but the 2019-nCoV result is still valid.

• When all controls exhibit the expected performance and the growth curves for the 2019-nCoV markers (N1, N2) AND the RNase P marker DO NOT cross the cycle threshold growth curve within 40.00 cycles (< 40.00 Ct), the result is invalid. The extracted RNA (obtained by direct method or with regular isolation) from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the re-tested sample is negative for all markers and RNase P, the result is invalid and collection of a new specimen from the patient should be considered.

• When all controls exhibit the expected performance and the cycle threshold growth curve for any one marker (N1 or N2, but not both markers) crosses the threshold line within 40.00 cycles (< 40.00 Ct) the result is inconclusive. The extracted RNA should be retested. If residual RNA is not available, re-extract RNA from residual specimen and re-test.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

10. Procedure Limitation

- The intended users must be trained with this technology prior to the use of this device.
- Any diagnostic results generated must be interpreted in conjunction with other clinical or laboratory findings.
- It is the intended user's responsibility to validate system performance for any procedures used in their laboratory which are not covered by Omixon Biocomputing Ltd.'s performance studies.
- Use this product only with following human biological samples: nasopharyngeal (NP) and oropharyngeal (OP) swabs
- The efficiency of the direct approach (without RNA extraction; heat treatment) is highly dependent on the VTM media used during the sample collection process. Using VTM from the following manufacturers: Clinichem, Copan, Puritan, CDC VTM is highly recommended. Other manufacturers' VTM, mwe Medical Wire must be validated by the end user laboratory.
- The direct approach may lead to a shift of the Ct value. Samples show a slightly higher Ct values (typically 1-2 cycles) while using the direct method. In rare cases the shift towards higher Ct values can account for up to 6 cycles. This may lead to a different positive, inconclusive or negative call in particular for samples being already in the high Ct range.
- A negative result does not exclude the possibility of infection, because results are dependent on appropriate specimen collection and absence of inhibitors. The presence of PCR inhibitors may cause invalid or inconclusive results which requires repetition.
- False Positive results may occur due to the several reasons, most of which relate to RNA contamination during specimen handling and preparation.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

11. Troubleshooting

Problems	Possible root cause	Recommendation
Fluorescent intensity is weak or does not appear in the positive control	Probe degradation	Use a new probe aliquot or repeat test with a new kit LOT
High inconsistency in the fluorescence signals in samples	Inaccurate pipetting	Use calibrated pipettes, make sure that an equal volume of reagents is added to each wells/tube
Fluorescent signal is detected in the negative control reaction	Carry over contamination	Always change tips between samples. Take care when dispensing samples, negative controls, and positive controls
	Contamination of the amplification master mix	Use a new aliquot of amplification mix
	Contamination of the extraction/preparation area	Use disinfectant to clean and disinfect the areas
No fluorescent signal is detected in all samples, including positive control	Probe degradation	Use new probe aliquot
	Thermal cycler setting error	Verify the correct program setting of the real time PCR instrument
	Wrongly prepared master mix, possible omitted component	Verify each component and repeat the PCR master mix preparation
False negative results	Presence of RT-PCR inhibitors	Validate the RNA extraction kit prior to use. Use VTM from the recommended manufacturers listed in this IFU
	Improper sample collection	Follow validated sample collection method
	Failure to follow instruction for use	Read the Instructions for Use carefully before processing samples. Any deviation from procedures written here may affect optimal performance.
	Using unauthorized reagents	Do not substitute or mix the AzureSeq kit reagents with reagents from other manufacturers.
False positive reaction	Cross contamination between samples	Take extra care when handle patient specimens, use straightforward and validated traceability processes in the laboratory.
	Sample mix-up	Discard the samples and repeat testing from new ones.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

12. Quality Control

Validating the whole assay procedure (direct or including a separate RNA extraction) and amplification session by processing a negative tested sample and a positive tested sample or a calibrated reference material) is recommended.

13. Performance characteristics

13.1. Analytical sensitivity - Limit of detection (LoD) studies

The LoD study established the lowest concentration of SARS-CoV-2 (genome equivalent copies(cp)/reaction) that can be detected by the N1 and N2 assays at least at 95% of the time using the AzureSeq CE reagents. LoD studies were performed on multiple real time PCR instruments.

13.1.1. BioRad CFX96 Touch

The LoD for the AzureSeq CE reagents for genomic RNA on the BioRad CFX96 Touch Real-time Detection System has been determined to be 5 copies per 20-µL reaction (or 0.25 copies/µL) for N1 and 10 copies per 20-µL reaction (or 0.5 copies/µL) for N2. Genomic RNA was spiked directly into PCR reactions and then analyzed on the BioRad CFX96 Touch Real-Time Detection System.

Genomic RNA on BioRAD CFX96 Touch Real-Time Detection System						
Targets	**N1**			**N2**		
RNA Conc. (copies/µL)	**0.05 (.2x)**	**0.25 (1x)**	**0.5 (2x)**	**0.05 (.1x)**	**0.5 (1x)**	**1 (2x)**
Positives/Total	**10/20**	**20/20**	**20/20**	**1/20**	**20/20**	**20/20**
Mean Ct (positives only)	**N/A**	**37.05**	**36.25**	**N/A**	**37.01**	**35.91**
Standard Deviation Ct	**N/A**	**0.68**	**0.55**	**N/A**	**0.5**	**0.33**

13.1.2. Applied Biosystems QuantStudio 3/5/7 Pro Real-time PCR System

The LoD for the AzureSeq CE reagents for genomic RNA on the Applied Biosystems QuantStudio 3 Real-time PCR System has been determined to be 10 copies per 20-µL reaction (or 0.5 copies/µL) for both N1 and N2 targets. Genomic RNA was spiked directly into PCR reactions and then analysed on the Quant-Studio Real-Time PCR System.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

A concordance study comparing the QuantStudio 3, 5 and 7 Pro platforms was conducted using 20 clinical specimens, previously identified as positive (n=11) and negative (n=9) for SARS-CoV-2. Mean Cts were calculated for N1, N2 and RNaseP for each instrument with the positive specimens while the Mean Ct for RNaseP was calculated for all negative specimens. These data demonstrated that there is no expected difference in clinical outcome across these platforms.

Genomic RNA on ThermoFisher QuantStudio 3 Real-Time PCR System								
Targets	**N1**				**N2**			
RNA Conc. (copies/µL)	**0.25**	**0.5**	**1**	**2**	**0.25**	**0.5**	**1**	**2**
Positives/Total	**16/20**	**20/20**	**20/20**	**20/20**	**15/20**	**19/20**	**20/20**	**20/20**
Mean Ct (positives only)	**32.04**	**31.12**	**30.45**	**29.04**	**38.67**	**37.02**	**36.47**	**34.35**
Standard Deviation Ct	**7.89**	**3.58**	**4.10**	**4.09**	**2.57**	**4.78**	**2.61**	**3.14**

13.1.3. Roche LightCycler® 480 System

The LoD for the AzureSeq CE reagents for genomic RNA on the Roche LightCycler® 480 real-time PCR System has been determined to be 10 copies per 20-µL reaction (or 0.5 copies/µL) for both N1 and N2 targets. Genomic RNA was spiked directly into PCR reactions and then analysed on the Roche LightCycler® 480 System.

Genomic RNA on Roche LightCycler® 480 System						
Targets	**N1**			**N2**		
RNA Conc. (copies/µL)	0,5	1	2	0,5	1	2
Positives/Total	22/23	23/23	23/23	23/23	23/23	23/23
Mean Ct (positives only)	37,63	36,76	35,78	36,37	35,55	34,61
Standard Deviation Ct	0,9062	0,5680	0,1956	1,795	0,4712	0,4441

13.2. Analytical specificity - Inclusivity testing

The AzureSeq CE system is using the primer/probe set for the N1 and N2 SARS-CoV-2 markers designed by the CDC which conducted the in-silico analysis on known sequences of SARSCoV-2. The data from this analysis are available in the FDA EUA EUA200001 "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

Results - *in silico* analysis of primer and probe sequences:

The oligonucleotide primer and probe sequences of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic Panel were evaluated against 31,623 sequences available in the Global Initiative on Sharing All Influenza Data (GISAID, https://www.gisaid.org) database as of June 20, 2020, to demonstrate the predicted inclusivity of the 2019-nCoV Real-Time RT-PCR Diagnostic Panel. Nucleotide mismatches in the primer/probe regions with frequencies > 0.1% are shown below. With the exception of one nucleotide mismatch with frequency > 1% (2.00%) at the third position of the N1 probe, the frequency of all mismatches was < 1%, indicating that prevalence of the mismatches were sporadic. Only one sequence (0.0032%) had two nucleotide mismatches in the N1 probe, and one other sequence from a different isolate (0.0032%) had two nucleotide mismatches in the N1 reverse primer. No sequences were found to have more than one mismatch in any N2 primer/probe region. The risk of these mismatches resulting in a significant loss in reactivity causing a false negative result is extremely low due to the design of the primers and probes, with melting temperatures > 60°C and with annealing temperature at 55°C that can tolerate up to two mismatches.

In Silico Inclusivity Analysis of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel Among 31,623 Genome Sequences Available from GISAID as of June 20, 2020

Primer/Probe	N1 probe	N1 reverse		N2 probe
Location (5’>3’)	3	15	21	13
Mismatch Nucleotide	C>T	G>T	T>C	C>T
Mismatch No.	632	34	71	46
Mismatch Frequency (%)	2.00	0.11	0.22	0.15

SARS-CoV-2 new variants

In silico analysis as well as RT-qPCR tests were performed against the currently known variants of SARS-CoV-2 virus: original alpha strain (WT), UK/SA (B.1.1.7), Delta (B.1.627.2), and Omicron variant (B.1.1.529). As predicted by the *in silico* analysis, AzureSeq reagents successfully amplify N1 and N2 targets against all tested RNA variants. N1 performance against the Omicron template, which contains a single mismatch in the N1 probe, was not affected and performed equally to the other variants.

In summary the AzureSeq reagents perform equally against all major strains of SARS-CoV-2 RNA seen in global circulation to the date of the issuance of this IFU (WT, B.1.1.7 UK/SA, B.1.627.2 Delta, B.1.1.529 Omicron). Therefore, it is expected that AzureSeq reagents will continue to amplify and detect SARS-CoV-2 Omicron variant RNA from individuals who have detectable levels of SARS-CoV-2 present in appropriately collected sample types.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

13.3. Analytical Specificity - Cross-reactivity

The AzureSeq CE is using the primer/probe set for the N1 and N2 SARS-CoV-2 markers designed by the CDC which conducted the cross-reactivity testing *in silico*. The CDC also conducted wet testing for cross-reactivity using the FDA Recommended List of Organisms. The data from these analyses is available in the FDA EUA EUA200001 "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostics Panel"

Result - specificity/exclusivity testing: *in silico* analysis

BLASTn analysis queries of the 2019-nCoV rRT-PCR assays primers and probes were performed against public domain nucleotide sequences. The database search parameters were as follows: 1) The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb; 2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry; 3) Database was updated on 10/03/2019; 4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000; 5) The match and mismatch scores are 1 and -3, respectively; 6) The penalty to create and extend a gap in an alignment is 5 and 2 respectively.

2019-nCoV_N1 Assay:

Probe sequence of 2019-nCoV rRT-PCR assay N1 showed high sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probe, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

2019-nCoV_N2 Assay:

The forward primer sequence of 2019-nCoV rRT-PCR assay N2 showed high sequence homology to Bat SARS-like coronaviruses. The reverse primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora. Combining primers and probe, there is no prediction of potential false positive rRT-PCR results.

In summary, the 2019-nCoV rRT-PCR assay N1 and N2, designed for the specific detection of 2019-nCoV, showed no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive rRT-PCR results.

In addition to the *in silico* analysis, several organisms were extracted and tested with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel to demonstrate analytical specificity and exclusivity. Studies were performed with nucleic acids extracted using the QIAGEN EZ1 Advanced

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

XL instrument and EZ1 DSP Virus Kit. Nucleic acids were extracted from high titer preparations (typically ≥ 105 PFU/mL or ≥ 106 CFU/mL). Testing was performed using the ThemoFisher Scientific TaqPath™ 1-Step RT-qPCR Master Mix, CG on the Applied Biosystems™ 7500 Fast Dx Real-Time PCR instrument. The data demonstrate that the expected results are obtained for each organism when tested with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel.

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

Specificity/Exclusivity of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel

Virus	Strain	Source	2019-nCoV N1	2019-nCoV N1	Results
Human coronavirus	229E	Isolate	0/3	0/3	Negative
Human coronavirus	OC43	Isolate	0/3	0/3	Negative
Human coronavirus	NL63	Clinical specimen	0/3	0/3	Negative
Human coronavirus	HKU1	Clinical specimen	0/3	0/3	Negative
MERS-coronavirus	-	Isolate	0/3	0/3	Negative
SARS- coronavirus	-	Isolate	0/3	0/3	Negative
bocavirus	-	Clinical specimen	0/3	0/3	Negative
Mycoplasma pneumoniae	-	Isolate	0/3	0/3	Negative
Streptococcus	-	Isolate	0/3	0/3	Negative
Influenza A (H1N1)	-	Isolate	0/3	0/3	Negative
Influenza A (H3N2)	-	Isolate	0/3	0/3	Negative
Influenza B	-	Isolate	0/3	0/3	Negative
Human adenovirus (1-es típus)	Ad71	Isolate	0/3	0/3	Negative
Human metapneumovirus	-	Isolate	0/3	0/3	Negative
Respiratory Syncytial Virus	Long A	Isolate	0/3	0/3	Negative
Rhinovirus	-	Isolate	0/3	0/3	Negative
Parainfluenza 1	C35	Isolate	0/3	0/3	Negative
Parainfluenza 2	Greer	Isolate	0/3	0/3	Negative
Parainfluenza 3	C-43	Isolate	0/3	0/3	Negative
Parainfluenza 4	M-25	Isolate	0/3	0/3	Negative

13.4. Microbial Interference Studies

Microbial interference studies are not required, as the *in-silico* analysis conducted by the CDC (FDA EUA EUA200001 “CDC 2019-Novel Coronavirus (2019- nCoV) Real-Time RT-PCR Diagnostics Panel”) did not meet the criteria that would require conducting interference studies.

13.5. Robustness test

The equivalence of the assay was compared with different enzyme master mixes.

Fifty (50) copies of genomic RNA is spiked into 20- µL reactions on the BioRad CFX96 Touch Real-Time Detection System. 12 positive samples are tested with each master mixes.

Tested enzyme master mixes:

- Thermo Fisher TaqPath 1-Step RT-qPCR System
- Promega GoTaq Probe-1 Step RT-qPCR System
- AzureSeq CE

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

Reagent Comparison: Genomic RNA spiked into PCR reactions on the BioRad						
Targets	**N1**			**N2**		
Master Mix	AzureSeq CE	Thermo Fisher TaqPath 1-Step RT-qPCR Master Mix, CG	Promega GoTaq Probe 1-Step RT-qPCR System	AzureSeq CE	Thermo Fisher TaqPath 1-Step RT-qPCR Master Mix, CG	Promega GoTaq Probe 1-Step RT-qPCR System
Positives/Total	12/12	12/12	12/12	12/12	12/12	12/12
Mean Ct	34.78	33.12	33.97	35.89	35.38	35.87
Standard Deviation Ct	0.31	0.24	0.20	0.27	0.25	0.16

13.6. Repeatability and reproducibility

Positive and negative samples were generated by adding positive and negative control into PCR reactions according to the Instructions for Use. 5 replicates of positive and negative samples were processed on QuantStudio 3 real time PCR instrument from Applied Biosystems by two operators to establish repeatability and reproducibility estimates for within run and operator to operator variability.

	Results by Operator 1				**Results by Operator 2**				**Agreement between operators (%)**
	N1	**N2**	**RNase P**	**Agreement between replicates (%)**	**N1**	**N2**	**RNase P**	**Agreement between replicates (%)**	
Positive samples	5/5	5/5	5/5	100	5/5	5/5	5/5	100	100
Negative samples	5/5	5/5	5/5	100	5/5	5/5	5/5	100	100

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

13.7. Clinical performance

Clinical oropharyngeal (OP) swab specimens previously determined by BioRad CFX96 to be positive (11 samples) or negative (6 samples) for SARS-CoV-2 were extracted on the KingFisher Flex System. Five (5) microliters of the eluted sample was added to the AzureSeq reagents and amplified and detected on the BioRad CFX96 system. All positive and negative samples showed 100% concordance with the original characterization by comparator.

Clinical nasopharyngeal (NP) samples (30 positive and 30 negative) were collected in VTM manufactured by Clinichem Ltd. (Hungary) and processed using the direct RNA extraction by heat treatment workflow of the protocol. Five (5) microliters of the heat-treated samples were added to the AzureSeq reagents and amplified and detected on the Roche LightCycler® 480 System. All positive and negative samples showed 100% concordance with the original characterization by comparator.

Platform	Sample type	Protocol	Results by AzureSeq CE		Results by Comparator		Concordance (%)
			Positive	Negative	Positive	Negative	
BioRAD CFX96	Clinical oropharyngeal sample (OP)	With RNA isolation	11	6	11	6	100
Roche LightCycler® 480	Clinical nasopharyngeal sample (NP)	Without RNA isolation	30	30	30	30	100

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

14. References

- 8/2003. (III.13.) ESzCsM rendelet az in-vitro diagnosztikai orvostechnikai eszközökről
- AzureSeq Validation Kit Functional Assay 96 well plate 20ul
- COMMUNICATION FROM THE COMMISSION Guidelines on COVID-19 in vitro diagnostic tests and their performance, Brussels, 15.4.2020 C(2020) 2391 final
- DIRECTIVE 98/79/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 27 October 1998 on in vitro diagnostic medical devices Working document of Commission services - Current performance of COVID-19 test methods and devices and proposed performance criteria 16 April 2020 (working document)
- ISO 13485:2016 - Medical devices - Quality management systems
- ISO 14971:2019 Medical devices — Application of risk management to medical devices
- Regulation (EC) No. 1907/2006 and Regulation (EC) No. 1272/2008
- BS EN ISO 23640:2015 In vitro diagnostic medical devices. Evaluation of stability of in vitro diagnostic reagents
- BS EN ISO 18113-1:2011 In vitro diagnostic medical devices. Information supplied by the manufacturer (labelling). Terms, definitions, and general requirements
- BS EN ISO 18113-2:2011 In vitro diagnostic medical Devices. Information supplied by the manufacturer (labelling). In vitro diagnostic reagents for professional use
- ISO 15223-1:2016 Medical devices — Symbols to be used with medical device labels, labelling and information to be supplied — Part 1: General requirements
- BS EN 13612:2002 Performance evaluation of in vitro diagnostic medical devices
- CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel, CDC-006-00019, Revision: 05, Effective: 07/13/2020
- ISO 20916:2019 In vitro diagnostic medical devices — Clinical performance studies using specimens from human subjects — Good study practice

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary

15. Symbols used on Labels

Lot number, identifies the reagent batch

Product code, identifies the IVD medical device

www.omixon.com

Consult electronic Instructions for Use

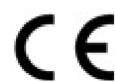
Contains sufficient reagents for <N> tests. The number accompained with this symbol Indicates the total number of tests that can be performed with the IVD medical device

Manufacturer, the date accompained with this symbol refers to the date of manufacture

Storage temperature, indicates the temperature limits to which the medical device can be safely exposed

Expiry date

In Vitro Diagnostic medical device



The European Conformity (en) or Conformité Européenne (fr) mark indicates compliance with 98/79/EC European Directive on in vitro diagnostic medical devices.

16. Contact information

For general assistance with this protocol contact:

Email Support: azureseq.support@omixon.com

Telephone Support: +36-70-672-7551

Manufacturer information:

Company name: Omixon Biocomputing Ltd
Visiting address: Kaposvár u. 14-18.
City: Budapest
Post code: H-1117
Country: Hungary

Copyright© 2022, Omixon Biocomputing Ltd. Confidential & Proprietary