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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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COVID-19 HT Screen
REF: RT-25HT
Detection of the COVID-19 genome with *Real Time PCR*

INTRODUCTION AND PURPOSE OF USE
The COVID-19 HT Screen system is a qualitative test that allows the identification, by means of *Real Time PCR*, of the *N* region (nucleocapsid phosphoprotein) of SARS-CoV-2 in subjects with suspected COVID-19 infection.
The Procedure allows the detection of the RNA target by means a retro-amplification reaction in a microplate.
The analysis of the results is made using a Real Time PCR analyzer instrument (thermal cycler integrated with a system for fluorescence detection).

CONTENT
The kit contains reagents enough to perform 96 amplification tests.

	Quantity	Description
R1	2 x 290 µl	**Amplification mMix** dNTPs, Tris-HCl, KCl, $MgCl_2$, Taq Polymerase, *AmpErase* Uracil N-Glycosylase (*UNG)* Nuclease-free water, ROX (Pink Cap)
R2	2 x 550 µl	**Covid-19 probe Mix** Upstream primers, downstream primers, Target probe N1 (FAM), Target probe N2 (VIC), Inhibition Control probe (Human RNase P - RP) (CY5) Nuclease-free water, (White Cap)
R3	2 x 35 µl	**N1-N2 Positive Control** synthetic RNA corresponding to N1-N2 region (Red Cap)
R4	2 x 50 µl	Negative Control

Instruction for use: **ST. RT25HT-ENG.1**

MATERIALS AND STRUMENTATION REQUIRED BUT NOT SUPPLIED
Disposable latex powder-free gloves or similar material;
Bench microcentrifuge (12,000 - 14,000 rpm);
Micropipettes and Sterile tips with aerosol filter;
Vortex;
Plastic materials (microplate and optical adhesive cover, Rotorgene tubes etc…);
Heat block (only for extraction)
ATL Buffer - Ref. 939016 - QIAGEN.
2 ml Microtube PP Skirted - Ref. 72.694 – QIAGEN
QIAsymphony plastic materials (Filter Tips, sample prep Cartriges, 8 Rod covers, etc…)

Reagents
The **COVID-19 HT Screen** kit was developed and validated to be used with the following extraction method:

Manual Extraction
Ref. 52906. QIAmp Viral RNA Mini Kit
The kit allows the manual RNA extraction from tested samples. The kit contains reagents for 250 samples. (QIAGEN)

Automatic Extraction
Ref.937036 QIAsymphony® DSP Virus/Pathogen kit.
The kit allows the automatic viral RNA from Human samples (Nasopharyngeal / oropharyngeal swabs and serum.). The kit contains reagents for 192/96 samples. (QIAGEN)

Instruments
The **COVID-19 HT Screen** kit was evaluated to be used with the following instruments:

Automatic Extraction
Ref. 9001297/9001301. QIAsymphony SP/AS.
Robotic Workstation for the automatic purification of the nucleic acids and plate setup simultaneously (QIAGEN).

Real Time PCR
- 7500 Fast from Lifetechnologies
- Rotor Gene Q MDx from QIAGEN
- CFX96 from Biorad

Please ensure that the instruments have been installed, calibrated, checked and maintained according to the manufacturers instruction and recommendations.

SAMPLES AND STORAGE
The **COVID-19 HT Screen** kit must be used with extracted RNA from biological samples: **Nasopharyngeal/oropharyngeal.** Collected samples must be shipped and stored at +2 - +8°C and used within 3 days from the collected data.
Store the sample at -20°C if used after 3 days.

PRECAUTIONS USE
This kit is for in *vitro* diagnostic (IVD), for professional use only and not for in vivo use.
After reconstitution, the amplification master mix must be used in one time. Repeat thawing and freezing of reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently. At all times follow Good Laboratory Practice (GLP) guidelines.
Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
Avoid any contact between hands and eyes or nose during specimen collection and testing.
Handle and dispose all used materials into appropriate bio-hazard waste containers. It should be discarded according to local law.
Keep separated the extraction and the reagents preparation.
Never pipette solutions by mouth.
Avoid the air bubbles during the master mix dispensing. Eliminate them before starting amplification.
Wash hands carefully after handling samples and reagents.
Do not mix reagents from different lots.
It is not infectious and hazardous for the health (see Material Safety data Sheet – MSDS).
Do not eat, drink or smoke in the area where specimens and kit reagents are handled.
Read carefully the instructions notice before using this test.
Do not use beyond the expiration date which appears on the package label.
Do not use a test from a damaged protective wrapper.

LIMIT OF THE METHOD
The extreme sensitivity of gene amplification may cause false positives due to cross-contamination between samples and controls. Therefore, you should:
- physically separate all the products and reagents used for amplification reactions from those used for other reactions, as well as from post-amplification products;
- use tips with filters to prevent cross-contamination between samples;
- use disposable gloves and change them frequently;
- carefully open test tubes to prevent aerosol formation;
- close every test tube before opening another one.

The proper functioning of the retro-amplification mix depends on the correct collection, correct transportation, correct storage and correct preparation of a biological sample.
As with any diagnostic device, the results obtained with this product must be interpreted taking in consideration all the clinical data and other laboratory tests done on the patient.
A negative result obtained with this product suggests that the RNA of SARS-CoV-2 was not detected in RNA extracted from the sample, but it may also contain SARS-CoV-2 RNA at a lower titre than the detection limit for the product (detection limit for the product, see paragraph on Performance Characteristics); in this case the result would be a false negative.
As with any diagnostic device, with this product there is a residual risk of obtaining invalid, false positives or false negatives results.

STORAGE AND STABILITY
Store the product **COVID-19 HT Screen** at –20°C.
The **COVID-19 HT Screen** kit is shipped on dry ice. The kit components should be frozen.
If one or more components are not frozen upon receipt or if the tubes have been compromised during transport, contact Clonit srl for assistance.
An intact and well stored product has a stability of 12 months from the date of production. Do not use beyond the expiration date which appears on the package label.
Repeat thawing and freezing of reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.

ANALYTICAL PROCEDURE
Manual Extraction
Ref. 52906. QIAmp Viral RNA Mini Kit
Follow the instructions inside the kit *QIAmp Viral RNA Mini Kit.*
Elute the sample in 50 µl of buffer AVE.

Automatic Extraction
Ref. 937055. QIAsymphony DSP Virus/Pathogen Kit
Follow the instructions inside the kit *QIAsymphony DSP Virus/Pathogen Midi Kit*
Use this volume of sample with 2 ml Microtube PP Skirted:

Nasopharyngeal / oropharyngeal swabs [µl] (including excess volume)
300

Select the protocol **Complex200_V6_DSP_default_IC**.

Preselected elution volume

Preselected elution volume (µl)	Initial elution volume (µl)
60	90

Preparation of the Carrier
To prepare a carrier RNA (CARRIER) stock solution, add 1350 µl Buffer AVE (AVE) (provided in 2µl vials) to the tube containing 1350 µg lyophilized carrier RNA (CARRIER) to obtain a solution of 1 µg/µl. Dissolve the carrier RNA (CARRIER) divide it into conveniently sized aliquots, and store at 2–8°C for up to 2 weeks.

Addition of the internal control to the samples
For Urine samples, 5µl internal controls must be added with carrier RNA(CARRIER)–Buffer AVE (AVE) mixture, and the total volume of the internal control–carrier RNA (CARRIER)–Buffer AVE (AVE) mixture remains 120 µl.

elution volume (µl)	RNA CARRIER (µl)	internal control (µl)	Buffer AVE (µl)	Final volume per sample (µl)
60	3	5	105	120

The CARRIER RNA solution will be added to the sample automatically by the instrument.

SOFTWARE SETTING
Lifetechnologies 7500 fast
Turn the instrument and the computer on and open the control software. Click on "**Advance Setup**": by default the software will shows the page "**experiment properties**". Write in the "**experiment name**" the file name, choose the type of instrument (**7500 o 7500fast),** the type of reaction (**quantitation standard curve**)**,** the type of used reagent (**Taqman®Reagents**) and the reaction time of analysis (**Standard ≈ 2 hours to complete a run**).
Open the page named "**page setup"** (sheet **Define Target and Samples**).

In the window **Define Targets** set:

Target mix Covid-19	Reporter	Quencher
N1	FAM	NONE
N2	VIC	NONE
Inhibition Control (RP)	CY5	NONE

Set the samples' name in the window "**Define Samples**".
In the same page "**plate setup**" select the sheet "**Assign Target and Samples**". On the screen you will see the microplate draft.

Choose an area of the plate where the Covid-19 mix will be placed and set the targets (N1, N2 and RP).
Select a well dedicated to N1-N2 Positive Control in the space "Assign target to selected wells" choose "Positive control".
Choose an area of the plate where the negative control will be placed. Select the "Negative (N) task" for the N1 and N2 and RP targets in the "Assign target to selected wells" space

Choose an area of the plate where the samples will be placed: select the wells of the plate and set the targets (N1, N2 and RP). Associate a sample being analyzed with each well using the "Assign samples to selected wells" window.
For each sample, select the "task UnKnown (U)" for the targets in the appropriate space "Assign targets to selected wells".

Set as passive reference, used as normalizer of the detected fluorescence, the ROX.

Open the "Run Method" page (sheet Graphic View) and set the correct thermal cycle by selecting "collect data" in the annealing phase at 55°C

cycles	UNG	Retrotrascription	Denaturation	Annealing
1	25°C 2 min	50° C 15 min		
1			95°C 2 min	
45			95° C 3 sec	55° C 30 sec

In the window "**Reaction volume plate per well**" set a volume of 20 µl.
After preparing the plate, and correctly inserting it in the instrument, press the button "**Start Run**".

Rotor Gene Q MDx
The experiments can be **set using the Quick Start Wizard or the** Advanced Wizard, which appears when the software is started.
Select the wizard "**Advanced**". As a first step, select the model "**Two Step Reaction**" with a double click in the "**New Run**".
In the next window, select the type of rotor installed on the instrument from the list that appears. Check the "**Locking Ring Attached**", check the checkbox and then click "**Next**".
Enter the name of the operator and the reaction volume of 20 µl, and then click "**Next**".
In the next window click on "**edit profile**". Set the following thermal cycle:

cycles	UNG	Retrotrascription	Denaturation	Annealing
1	25°C 2 min	50° C 15 min		
1			95°C 2 min	
45			95° C 3 sec	55° C 30 sec

Select the annealing from the thermal profile and click on "**Acquiring A to cycling**."
In the next window, select Yellow and Red from the available channels and add it to **acquiring channel** along with the Green channel and click "**OK**".
Click on "Edit Gain" button and set the following values for each channel:

Target	Reporter	Gain
N1	Green	5
N2	Yellow	5
RP	Red	5

In the next window click on "**OK**" and then click "**Next**".
To begin the course, click on the button "**Start Run**". You can save the model before you begin your run by clicking on "**Save Template**".
After clicking on the button "**Start Run"** window appears "**Save As**". The stroke can be saved in the desired position by the user.
Once the run started, the window "**Edit Samples**" allows you to set the name of samples and controls in the positions in which they were loaded on the instrument.
Select the position where N1-N2 Positive Control has been placed and name it. By clicking on the corresponding "Type" box, in the "Samples" drop-down menu it is possible to select the type of sample being analyzed. Select Positive Control.
Select the position where the Negative Control was placed and name it as Negative Control. By clicking on the corresponding "Type" box, in the "Samples" drop-down menu it is possible to select the type of sample being analyzed. Select "Negative Controls"
Select the position of each individual sample and enter the patient name or code. By clicking on the corresponding "Type" box, in the "Samples" drop-down menu it is possible to select the type of sample being analyzed. Select "UnKnown"
At the end of the operation click "OK" in the "edit samples" window and wait for the end of the run for the analysis (see "Interpretation of results").

CFX96 Real Time PCR
Turn the instrument and the computer on and start the control software. In the principal screen will appear the window "**Startup wizard**": select "**CFX96"** and press **"ok".** In the next window push "**create new**" and set the thermal protocol and the reaction volume (20µl):

cycles	UNG	Retrotrascription	Denaturation	Annealing
1	25°C 2 min	50° C 15 min		
1			95°C 2 min	
45			95° C 3 sec	55° C 30 sec

Save the protocol and click "**Next**". The software will open in default the sheet "**plate**". Click "**create new**", select **"Fluorophores button"** to choose fluorophores (FAM, VIC and CY5).

Target mix Covid-19	Reporter	Quencher
N1	FAM	NONE
N2	VIC	NONE
Inhibition Control (RP)	CY5	NONE

Select the well containing the N1-N2 Positive Control and choose "Sample Type" from the drop-down menu: Positive Control Click "Load check boxes" to load the chosen fluorophores and write or select the Target Name
Select the wells containing the negative control and choose from the drop-down menu "Sample Type": NTC. Click "Load check boxes" to load the chosen fluorophores and write or select the Target Name.
Select the wells containing the samples under test and choose from the drop-down menu "Sample Type": Unknown. Click "Load check boxes" to load the chosen fluorophores and write or select the Target Name. Save the plate by clicking the "Next" button and, as soon as the plate has been prepared and correctly inserted into the instrument, press the "Start Run" button.

PREPARATION OF THE REACTIONS:
Manual Procedure
Defrost a tube of Amplification Mmix;
Defrost a tube of Covid-19 probes Mix;
Mix carefully by vortex 275µl of Amplification Mmix directly into the 550 µl tube of Covid-19 probes Mix (the mixture thus produced is enough to prepare 48 reactions: 1 positive control N1-N2, 1 negative control and 46 samples).
Distribute, in the amplification plate, **15µl** of just reconstituted mix in chosen positions as already set on the instrument software.
Add 5µl of the solution taken from the negative control vial in the negative control position.
Distribute, in chosen positions for each sample, 5µl of the corresponding sample.
Distribute in chosen positions for the positive control: 5µl of N1-N2 positive control.
Carefully seal the plate using optichal adhesive films and check that, in the mix, there are no air bubbles that could interfere with the amplification.
For the Rotor-geneQ 5/6 plex instrument, carefully seal each tube with the appropriate caps. The presence of air bubbles are not influential: the centrifugal force of the rotor will allow their automatic elimination.
Transfer the plate to the instrument and press the "Run" button.

QIAsymphony AS Plate Setup.
Samples processed on the QIAsymphony SP can be transferred automatically to the QIAsymphony AS (integrated operation) to the Rotor-Gene Q tubes or 96 wells MicroPlate
Defrost a tube of **Amplification mMix**;
Defrost a tube of di **Covid-19 probe Mix;**
Mix carefully by vortex 275µl of Amplification Mmix directly into the 550 µl tube of Covid-19 probes Mix (the mixture thus produced is enough to prepare 48 reactions: 1 positive control N1-N2, 1 negative control and 46 samples).
Insert the thus obtained Master Mix tubes in the correct position inside the QIAsymphony AS;
Defrost the N1-N2 positive controls and insert it in the chosen positions of **QIAsymphony** Defrost a tube of **Negative Control** and insert it in the chosen positions of **QIAsymphony AS**;

Follow the instructions of *QIAsymphony AS.*
Select the Assay Parameter Set to use in the run

Assay Parameter Set
APS_15+5 (user-prep_MM)

After the run is finished, press "Remove" in the assay setup"Overview" screen. Open the "Assays" drawer and unload the assayrack(s). Download the result and cycler files and proceed to protocol on the Real Time Instrument (RotorGene Q MDx).

QUALTITATIVE ANALYSIS
Lifetechnologies 7500 Fast
At the end of the PCR run, the software automatically opens the "**Analysis**" window in the "**Amplification plot**" sheet on the menu on the left.
Select the wells corresponding to the positive control, negative control and samples for analysis.
Select in the "**Option**" window inside the "**Target**" pop-up menu the **N1 target**. Check the correct setting of the threshold.
Select in the "**Option**" window inside the "**Target**" pop-up menu the **N2 target**. Check the correct setting of the threshold.
Select in the "**Option**" window inside the "**Target**" pop-up menu the **RP target**. Check the correct setting of the threshold.

The analysis of the results is made selecting from the menu in the left the page **"Analysis"**. From the page "**Standard Curve**", maintaining open the sheet "**view well plate**" in the right side of the software select the wells containing the points of the curve and verify the parameters described in the paragraph "**Interpretation of the Results**" (coefficient of correlation, slope etc).
From the page "**Amplification Plot**" verify the amplification plot for every single sample.
Opening the sheet "**view well table**" in the right side of the software it is possible to verify the data obtained from experiments: Threshold Cycles and emitted fluorescence.
Clicking from the menu file and selecting the box export, the window "**export properties**" will open. Indicate the file name, select the position to save it (**Browse**) and click on button **"Start export".** In this way the software will permit to save a excel file with all the data corresponding to selected experiment.

Rotor Gene Q MDx
At the end of the PCR run open the "**Analysis**" window. Select the "**Quantification**" sheet and click on "**cycling A (green)".**
Select from the menu **"Dynamic Tube"** and subsequently **"Slope correct"**.
Check the correct setting of the threshold in the space provided "**CT calculation – Threshold"**.
Open the "**Analysis**" window. Select the "**Quantification**" sheet and click on "**cycling A (yellow)".**
Select from the menu **"Dynamic Tube"** and subsequently **"Slope correct"**.
Check the correct setting of the threshold in the space provided "**CT calculation – Threshold"**.

Also in this case, you can print a report of the analysis by clicking on the **"Report"** window and selecting the file in the first **Quantification cycling A (green)** and then the file **cycling A (yellow) and cycling A (red).**

CFX96 Real Time PCR System
At the end of the PCR, select the "**quantitation**" sheet. On the top of the screen, select "**settings**" from the menu and choose "**Baseline Threshold**" for each fluorophore.
For more accurate results, is essential to set threshold for each fluorophore separately of the different amplification mix.
You can export the report by selecting the paper block figure on the top of the screen.

INTERPRETATION OF RESULTS
In the amplification reactions of each sample, the Ct values of each specific probe for N1 and N2 are used to detect the presence of the Target being analyzed.
The fluorescence increase of the specific probes for N1 (FAM) and N2 (VIC) indicate the ability of the test to detect SARS-CoV-2 RNA.

Target	IC	Interpretazione
Ct N1 and / or N2 < 40	Ct not relevant	**RNA SARS-CoV-2 Detected**
Ct and / or N2 > 40	Ct < 35	**Doubtful result** *Repeat the test from the extraction step. If the data is confirmed, the sample is considered weak positive*
Ct N1 and N2 Absent	Ct < 35	**RNA SARS-CoV-2 Not Detected**
Ct N1 and N2 Absent	Ct > 35	**Invalid Result** *Extraction and/or swab not performed correctly. Proceed by repeating the test from the extraction phase. If the data is confirmed inconclusive, repeat the execution of the buffer.*

As with any diagnostic device, the results obtained with this product must be interpreted taking in consideration all the clinical data and the other laboratory tests done on the patient.
As with any diagnostic device, with this product there is a residual risk of obtaining invalid, false positives or false negatives results.

The use of positive and negative controls in each amplification session allow to verify the correct functioning of the amplification mix and the absence of any contamination.
The instruments' software is able to analyze the emitted fluorescence of specific probes for N1 (FAM), N2 (VIC) and the specific probe of the internal control RP (Cy5).

A correct functioning of the amplification mixture must be verified by analyzing the following parameters:

Parameter	Reference
N1 positive Control	Ct ≤ 34
N2 positive Control	Ct ≤ 34

If the results of the amplification reaction of the positive controls produce a Ct> 34 or undetermined, the session cannot be considered valid and must be repeated.

The results obtained with this product must be interpreted taking in consideration all the clinical data and the other laboratory tests performed on the patient.

PERFORMANCES

Analytical sensitivity:

It is considered as analytical sensitivity the highest dilution (title) to which a positive sample can be diluted without the system losing the ability to detect with a positivity rate of ≥ 95%. The analytical sensitivity of the system was assessed by analyzing synthetic RNA, quantified by spectrophotometric analysis, containing the regions of interest of the virus in serial dilutions.

Copies/ul N1	95% confidence interval N1
9,84 cps/ul	Inf. 6,97 cps/ul Sup. 18,72 cps/ul

Copies/ul N2	95% confidence interval N2
6,51 cps/ul	Inf. 4,66 cps/ul Sup. 11,53 cps/ul

The analytical sensitivity of ***COVID-19 HT Screen*** kit is determined by Probit analysis.

Clinical sensitivity:

It is considered as clinical sensitivity the ability to detect true positive samples in the totality of the samples screened as positive. The analysis was made on SARS-CoV-2 positive samples and the test was performed following the method recommendations. Positive samples were confirmed with an other method available on the market.

Obtained results show a clinical sensitivity of 100%: for RNA samples extracted.

Diagnostic Specificity:

It is considered as diagnostic specificity the ability of the method to detect trues negative samples. The diagnostic specificity of the system was evaluated analyzing human samples tested and confirmed as SARS-Cov-2 negative with an other method available on the market.

Diagnostic specificity is 100% for RNA samples extracted.

Analytical Specificity:

Test's specificity was guaranteed by the use of specific primers for SARS-CoV-2
The alignment of the chosen regions for specific primers hybridization for SARS-CoV-2 with available sequences of the region present in database, demonstrated: their conservation, the absence of significative mutations for the analysed target.

Cross-Reactivity:

The exam of alignment of the region chosen for hybridization of primers specific for SARS-CoV-2 with the sequences available in the bank data of the N region showed their conservation, the absence of significant mutations and the complete specificity for the targets analysed.
To check the cross-reactivity of the assay, samples tested as positive for other viruses were analysed following the method instructions.

Sample	Positive sample	Obtained Results cps/ml
1	Other CoronaVirus Isolates	< 100
2	Other CoronaVirus Isolates	< 100
3	Other CoronaVirus Isolates	< 100

INTERFERENCES:

Verify that in the RNA extracted from the sample there is no contamination from mucoproteins and haemoglobin, to exclude possible inhibition of PCR reaction. The interference due to contaminants can be detected through a spectrophotometric analysis, verifying the ratio between the absorbance readings at 260 nm (maximum absorbtion of Nucleic Acids) and 280 nm (maximum absorbtion of Proteins). A pure RNA should have a ratio of approximately 2.

QUALITY CONTROL

It is recommended to include in each analytical run, as quality control of every extraction, amplification and detection step, an already tested negative and positive sample, or a reference material with known concentration.
In accordance with the Clonit srl ISO EN 13485 Certified quality Management System, each lot of **COVID-19 HT Screen** is tested against predetermined specification to ensure consistent product quality.

BIBLIOGRAPHY

Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases Interim guidance 17 January 2020 WHO/2019 nCoV/laboratory/2020.3

CDC/NCIRD/DVD Effective: 24 Jan 2020

TECHNICAL ASSISTANCE

For any question and support please contact our Technical support:

e-mail: info@clonit.it
phone: +39 02 56814413

IVD	*In vitro* diagnostic device
	Read the instruction's manual
	Range of temperature
	Use within (dd/mm/yyyy: year-month)
LOT	Lot (xxxx)
REF	Code
	Manufacturer
Σ	Contains sufficient for <n> tests

EDMA 15044019
CND: W0105040519

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CE IVD
for *in vitro* diagnostic use

ST. RT25HT-ENG.1
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