

# **Ezplex SARS-CoV-2 G Kit**

For Emergency Use Authorization (EUA) only.  
For *in vitro* diagnostic use only.  
For prescription use only.

## **Instructions for Use (IFU)**



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## Proprietary Name: Ezplex SARS-CoV-2 G Kit

### I. Intended Use

The Ezplex SARS-CoV-2 G Kit is a real-time RT-PCR *in vitro* diagnostic test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, and sputum specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 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 Ezplex SARS-CoV-2 G Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The Ezplex SARS-CoV-2 G Kit is only for use in the United States under the Food and Drug Administration's Emergency Use Authorization.

### II. Summary and Explanation

Coronaviruses are a large family of viruses which may cause illness in animals or humans. In humans, several coronaviruses are known to cause respiratory infections ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The most recently discovered coronavirus, SARS-CoV-2, causes the associated coronavirus disease COVID-19. This new virus and disease were unknown before the outbreak

began in Wuhan, China, in December 2019.<sup>1</sup>

The most common symptoms of COVID-19 are fever, tiredness, and dry cough. Some patients may have aches and pains, nasal congestion, runny nose, sore throat, new loss of taste or smell, or diarrhea. These symptoms are usually mild and begin gradually. Some people become infected but don't develop any symptoms and don't feel unwell. The disease can spread through respiratory droplets produced when an infected person coughs or sneezes. These droplets can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs.<sup>2</sup> These droplets also can land on objects and surfaces around the person.<sup>3</sup> Other people may acquire SARS-CoV-2 by touching these objects or surfaces, then touching their eyes, nose, or mouth.

The virus that causes COVID-19 is infecting people and spreading easily from person to person. On March 11, 2020, the COVID-19 outbreak was characterized as a pandemic by the World Health Organization (WHO).<sup>4,5</sup>

### III. Principles of the Procedure

The Ezplex SARS-CoV-2 G Kit uses TaqMan-based real-time reverse transcription polymerase chain techniques to conduct in vitro reverse transcription of SARS-CoV-2 RNA, DNA amplification and fluorescence detection. The assay targets specific genomic regions of the SARS-CoV-2 RdRP and N genes. Nucleic acid is isolated and purified from respiratory specimens using the Qiagen QIAamp® DSP Viral RNA Mini Kit. The purified nucleic acid is then reverse transcribed into cDNA using the Ezplex SARS-CoV-2 G kit. The cDNA is then subsequently amplified using either the CFX96 Dx Real-time PCR instrument (Bio-Rad) or the Applied Biosystems 7500 Real-time PCR instrument (ThermoFisher Scientific). During this 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, 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.

The negative control included in the kit serves as a general control for exogenous nucleic acid contamination and is used to monitor cross-contamination during the RNA extraction and PCR reaction setup steps. It should be run with each batch of tests.

The positive control included in the kit consists of synthesized plasmid DNA for each gene target and is used to monitor for the presence of inhibitors and the efficiency of the polymerase chain reaction. It should be run with each batch of tests.

An internal control is utilized to ensure that clinical specimens are successfully amplified and detected. This control consists of plasmid DNA that was synthesized to include a portion of the human BCR activator of RhoGEF and GTPase (BCR) gene. This control is added to the reaction master mixture during PCR preparation procedure.

The Ezplex SARS-CoV-2 G Kit does not include an internal control for RNA extraction/recovery or transcription. A known SARS-CoV-2 positive specimen or specimen containing SARS-CoV-2 RNA (i.e., in vitro transcript or pseudovirus) must be tested with every batch of patient specimens to monitor the integrity of these process steps.

#### IV. Kit Components and Packaging Specifications

##### Catalog Number: GNT2011-1 100 tests/kit

No.	Component Name*	Volume	Main Ingredients
1	RQ Mixture	1 vial, 1000uL	DNA Taq polymerase, Reverse Transcriptase, dNTPs with dUTP, Magnesium Chloride, Potassium, Uracil N-glycosylase
2	P+P	1 vial, 500uL	Tris-HCl, EDTA, Oligonucleotide primers specific for SARS-CoV-2 virus, Fluorescent-labeled oligonucleotide probe specific for SARS-CoV-2 virus
3	Positive Control (PC)	1 vial, 50uL	Tris-HCl, EDTA, Synthesized DNA control specific for SARS-CoV-2 virus
4	Negative Control (NC)	1 vial, 50uL	Double Distilled Water
5	Internal Control (IC)	1 vial, 20uL	Tris-HCl, EDTA, Synthesized DNA internal control

\*RQ Mixture: Real-time Quantitative PCR Mixture; P+P : Probe + Primer

##### Catalog Number: GNT-2011-2 200 tests/kit

No.	Kit Component*	Volume	Main Ingredients
1	RQ Mixture	2 vials, 1000uL	DNA Taq polymerase, Reverse Transcriptase, dNTPs with dUTP, Magnesium Chloride, Potassium, Uracil N-glycosylase
2	P+P	2 vials, 500uL	Tris-HCl, EDTA, Oligonucleotide primers specific for SARS-CoV-2 virus, Fluorescent-labeled oligonucleotide probe specific for SARS-CoV-2 virus
3	Positive Control (PC)	2 vials, 50uL	Tris-HCl, EDTA, Synthesized DNA control specific for SARS-CoV-2 virus

4	Negative Control (NC)	2 vials, 50uL	Double Distilled Water
5	Internal Control (IC)	2 vials, 20uL	Tris-HCl, EDTA, Synthesized DNA internal control

**\*RQ Mixture: Real-time Quantitative PCR Mixture; P+P : Probe + Primer**

**Optional Materials Provided:**

- Genetree Viewer Software (CAT No. GNT2011-3)

## V. Materials Required But Not Provided

No.	Name	CAT No.	Company
1	For Bio-Rad instrument: any applicable 96 well PCR plate plastics for CFX96 Dx system	MLL9651	Bio-Rad
2	For Bio-Rad instrument: any applicable PCR plate sealing film for CFX96 Dx system	MSB1001	Bio-Rad
3	For ThermoFisher Scientific instrument: microAmp Optical Adhesive Film	4311971	ThermoFisher Scientific
4	For ThermoFisher Scientific instrument: microAmp Optical 96-well Reaction Plate	N8010560	ThermoFisher Scientific
5	For ThermoFisher Scientific instrument: microAmp Optical 8-tube strip(0.2mL)	431567	ThermoFisher Scientific
6	For ThermoFisher Scientific instrument: microAmp Optical 8-cap Strip	4323032	ThermoFisher Scientific
7	Qiagen QIAamp® DSP Viral RNA Mini Kit	61904	Qiagen
8	Known SARS-CoV-2 positive specimen or specimen containing SARS-CoV-2 RNA(in vitro transcript or pseudovirus) to control for extraction/recovery or transcription	MBC137-R (Recommended)	Vircell
9	Computer for installation of Genetree Viewer Analysis Software	1) Microprocessor: Intel(R) i3 3.5 GHz or above 2) Memory: 4 GB or larger; 3) Microsoft Windows 7 or above; 4) more than 1 USB port	

## VI. Instruments

PCR Instruments validated for use with the Ezplex SARS-CoV-2 G Kit:

- CFX96 Dx Real-time PCR Instrument with 1.6 or 3.1 or later versions of CFX Manager (Bio-Rad) **OR**
- ABI 7500 Real-time PCR Instrument with 2.3 or later versions of 7500 software (ThermoFisher Scientific)

## VII. Storage and Handling Conditions

- All kit materials should be stored at -20 °C opened and unopened.
- Use the reagents before the expiration date shown on the labeling.
- Completely thaw the reagents before use.
- Repeated thawing and freezing should be avoided. It should not exceed 5 freeze-thaw cycles.

## VIII. Warnings and Precautions

- For *in vitro* diagnostic use. For use under an Emergency Use Authorization (EUA) only.
- This product has not been FDA cleared or approved; but has been authorized by the FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories; laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. §360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim
- Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV. <https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html>.
- Specimens may be infectious. Use Universal Precautions when performing this assay. Proper handling and disposal methods should be established by the laboratory director. Only personnel adequately trained in handling infectious materials should be permitted to perform this diagnostic procedure.<sup>6</sup>
- Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of being infected with SARS-CoV-2 as outlined in CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019 Novel Coronavirus (2019-nCoV).
- Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling

specimens and reagents.

- Dispose of all material that has come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.
- Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.
- Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of virus or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens. It is recommended to use sterile disposable filter-tips to aspirate reagents and specimens.
- Do not use the reagents and controls after the expiration date.
- Do not mix reagents from different lots.
- Since the plasmid DNA in the positive control can degrade, it is recommended that the reagents shall be divided into amounts required for 1-2 tests and stored in a freezer.
- Only the Qiagen QIAamp® DSP Viral RNA Mini Kit can be used with the Ezplex SARS-CoV-2 Kit for nucleic acid extraction.
- Only the Bio-Rad CFX Dx Real-time PCR Instrument and the ThermoFisher Scientific ABI 7500 Real-time PCR Instrument can be used with the Ezplex SARS-CoV-2 Kit. These instruments should be calibrated regularly according to instrument's instructions to eliminate cross-talks between channels.
- The Ezplex SARS-CoV-2 Kit uses PCR-based technology and testing should be conducted in three separate areas: reagent preparation area, specimen preparation area and amplification area. Protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.) should be worn during operation and protective equipment accessories should be changed when entering and leaving different work areas. Protective equipment accessories in each work area are not interchangeable.
- Store assay components at the recommended storage condition.
- Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.
- Contamination may occur if carryover of specimens is not adequately controlled during specimen pool preparation, handling, and processing.

## IX. Collection, Storage and Shipment of Specimens

Only upper respiratory specimens collected in VTM (such as nasopharyngeal and oropharyngeal swabs) and sputum specimens can be used with the test.



### A. Specimen Collection

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. Place swabs immediately into sterile tubes containing 3 ml of viral transport media (VTM). For initial testing, nasopharyngeal swab specimens are recommended. Collection of oropharyngeal swabs is a lower priority and is acceptable if other swabs are not available.

- Nasopharyngeal swab (NP): Insert a 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.
- Oropharyngeal swab (e.g., throat swab, OP): Swab the posterior pharynx, avoiding the tongue.
- Sputum: Educate the patient about the difference between sputum and oral secretions. Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile screw-cap collection cup or sterile dry container.

### B. Specimen Storage

A sample collection device is not a part of the assay kit. Patient samples must be collected according to appropriate laboratory guidelines. All testing for COVID-19 should be conducted in consultation with a healthcare provider. We recommend using CDC guidelines for sample collection of respiratory specimens and sample storage (<https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>).

Specimens should be processed within 48 hours from collection and stored at 2-8°C during that time. If the specimens cannot be tested within 48 hours, samples should be stored frozen at -70°C or colder.

### C. Shipping

Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation External Icon. Store specimens at 2-8°C and ship overnight to the lab on ice pack. If a specimen is frozen at -70°C ship, overnight to the lab on dry ice. Additional useful and detailed information on packing, shipping, and transporting specimens can be found at Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).

### D. For more information, refer to:

Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from

Persons for Coronavirus Disease 2019 (COVID-19)  
<https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html>  
 Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens  
 Associated with Coronavirus Disease 2019 (COVID-19)  
<https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html>

## E. Specimen Preparation

### 1. Specimens for Individual Testing

- Nucleic acid should be isolated and purified from nasopharyngeal swabs, and oropharyngeal swabs, and a sputum specimens using the QIAamp® DSP Viral RNA Mini Kit.
- Prior to the start of the nucleic acid extraction, an additional negative clinical sample should be prepared and spiked with a known SARS-CoV-2 control. This functions as an extraction control to assess extraction reagent integrity and successful RNA extraction.
- Ensure homogenous mixing of prepared specimens.
- Utilize 140 µL of clinical sample and elute with 50 µL of Buffer AVE from the QIAamp® DSP Viral RNA Mini Kit. If the extracted RNA cannot be used immediately, store at 2 to 8 ° C for up to 24 hours or at -70 ° C for up to 1 month.
- Refer to the QIAamp® DSP Viral RNA Mini Kit Handbook for the protocol for extracting RNA using the QIAamp® DSP Viral RNA Mini Kit.

## X. Test Procedure

### A. Nucleic Acid Extraction

The QIAamp® DSP Virus Viral RNA mini Kit (Qiagen GmbH) must be used for RNA extraction and users shall follow the protocol included in the Kit Instructions for Use. After extraction, RNA if not used immediately should be divided into amounts required for 1-2 tests and stored at -70°C since RNA can degrade.

### B. Real-time PCR Amplification

#### 1. Reagent Master Mix Solution Preparation

a. Refer to the table below and prepare the PCR master mix solution according to the number of specimens/controls to be tested.

Component	Volume (uL) per Specimen/Control
RQ Mixture (Real time Quantitative Mixture)	10
P + P (Probe + Primer)	5
Internal control	0.1

Total	15.1
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b. Pipette 15uL of PCR master mix solution into each 96-well PCR plate or 8-cap strip. Add 5uL of the extracted RNA specimen into each 96-well PCR plate or 8-cap strip.

c. Also, add 5 uL each of the positive control and negative control into each 96-well PCR plate or 8-cap strip.

d. If the 96-well PCR plate is used for the preparation, seal the top of the plate thoroughly to prevent the liquid from spilling or leaking.

e. If the 8-cap strip is used for the preparation, make sure that every cap is tightly closed on the top of the strips.

f. Centrifuge the 96-well PCR plate or 8-cap strip to make sure that all liquids are placed at the bottom.

g. Each batch of samples tested should include the following controls: positive control, negative control, and extraction/transcription control.

**2. PCR Instrument Set Up**

a. Set up the Bio-Rad or Applied Biosystems PCR Instrument according to their respective Instrument Reference Guide/Manual using the cycling specifications below:

Step	Temperature / Time	Cycle
Hold	25 °C / 2 min	1 Cycle
	50 °C / 30 min	
	95 °C / 5 min	
Cycle	95 °C / 15 sec	40 Cycles
	60 °C / 45 sec	

b. Set up fluorescent thresholds for detection targets per the table below.

c. The threshold should be adjusted to fall within the exponential phase of the fluorescence curves and above any background noise signal. The procedure chosen for setting the threshold should be used consistently.

Device	FAM	Cy5	VIC(HEX)
Bio-Rad CFX96 Dx	500	250	500
ThermoFisher Scientific AB7500	0.4	0.1	0.05

d. Add the prepared 96-well PCR plate or 8-cap strips to the PCR instrument and run the Bio-Rad or Applied Biosystems PCR Instrument according to their respective Instrument Reference Guide/Manual.

## XI. Quality Control

A Positive Control and Negative Control are provided with the Kit and should be run with each batch of specimens. An internal control [plasmid DNA synthesized to include a portion of the human BCR activator of RhoGEF and GTPase (BCR) genes] is provided with the kit and is utilized to ensure that each clinical specimen is successfully amplified and detected. This control is added to the reaction master mix during PCR preparation procedure. The Ezplex SARS-CoV-2 G Kit does not include an internal control for RNA extraction/recovery or transcription. A known SARS-CoV-2 positive specimen or specimen containing SARS-CoV-2 RNA (i.e., in vitro transcript or pseudovirus) must be tested with every batch of patient specimens to monitor the integrity of these process steps.

## XII. Genetree Viewer Software Analysis

**NOTE: Please contact 'technicalsupport@smlgenetree.com' to acquire 'Genetree Viewer' software prior to running the Ezplex SARS-CoV-2 G Test. Please refer to the Genetree Viewer Software Operators Manual for more detailed information.**

### A. General Description of the Software and Example Screens

No.	Description
①	Positive/Negative results by well are indicated in '+', '-' respectively. Invalid results by well are indicated in ' ' respectively. Inconclusive results by well are indicated in '?' respectively.
②	Ct and fluorescent values of the results for each well are plotted on a graph.
③	Ct values of the results for each well are indicated numerically and qualitative results are printed.
④	Analysis results are converted into an excel spreadsheet.



No.	Sample ID	Sample	Well	Content	RdRp	N	IC	Result	Validity	Comment
10			B02		36,06	36,97	27,75	Positive	Valid	
11			C02		36,96	38,28	27,68	Positive	Valid	
12			D02		39,14	37,61	27,62	Positive	Valid	
13			E02		39,04	N/A	27,51	Inconclusive	Valid	
14			F02		N/A	N/A	27,50	Negative	Valid	
15			G02		N/A	N/A	27,41	Negative	Valid	
16			H02		N/A	N/A	N/A	Invalid	Invalid	Retest after re-extraction
17			A03		36,12	37,22	27,88	Positive	Valid	
18			B03		35,71	36,67	27,69	Positive	Valid	

### XIII. Interpretation of Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

The table below lists the expected results for the kit with valid positive control and negative control.

FAM Ct (RdRp)	CY5 Ct (N)	VIC/HEX Ct (IC)	Result*	Comment
<40	<40	Any	<b>Positive</b>	SARS-CoV-2 RNA is detected
<40	Neg	Any	<b>Inconclusive</b>	Further confirmatory testing may be conducted if clinically indicated
Neg	<40	Any	<b>Inconclusive</b>	Further confirmatory testing may be conducted if clinically indicated
Neg	Neg	<38	<b>Negative</b>	SARS-CoV-2 RNA is not detected
Neg	Neg	≥38 or Neg	<b>Invalid</b>	Retest after re-extraction

- The result is judged as Positive only when both the RdRp and N genes are detected.
- Further confirmatory testing may be conducted if clinically indicated if the result is judged as "Inconclusive".
- Retesting after re-extraction is necessary if the result is judged as "Invalid." If the re-tested result remains invalid, report the invalid result and consider collecting a fresh sample and re-testing if clinically indicated.

### XIV. Limitations

- The Ezplex SARS-CoV-2 G Kit has been analytically validated for use with nasopharyngeal and oropharyngeal swabs in VTM and sputum specimens run on the Bio-Rad CFX96 Dx Real-Time PCR and ABI 7500 Real-time PCR Instrument and utilizing the Qiagen QIAamp® DSP Viral RNA Mini Kit for RNA extraction. This assay has been clinically validated for use with nasopharyngeal/oropharyngeal swabs in VTM and sputum specimens run on the Bio-Rad CFX96 Dx Real-Time PCR utilizing the Qiagen QIAamp® DSP Viral RNA Mini Kit for RNA extraction.

- Based on the *in-silico* analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the N gene in the Ezplex assay. SARS-CoV is not known to be currently circulating in the human population, and therefore is unlikely to be present in patient specimens.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- The procedures in this handbook must be followed, as described. Any deviations may result in assay failure or may cause erroneous results.
- Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
- Reliable results are dependent on adequate specimen collection, transport, storage, and processing.
- Good laboratory practices are required to ensure the performance of the kit, with care required to prevent contamination of the kit components. Components should be monitored for contamination and any components thought to have become contaminated should be discarded as standard laboratory waste in a sealed pouch or zip-lock plastic bag.
- All specimens should be handled as if they are infectious following proper biosafety precautions.
- False negative results may be caused by:
  - Unsuitable collection, handling and/or storage of specimens
  - Specimen outside of viremic phase
  - Failure to follow procedures in this handbook
  - Use of unauthorized extraction kit or PCR platforms
- False positive results may be caused by:
  - Unsuitable handling of specimens containing high concentration of COVID-19 viral RNA or positive control template
  - Unsuitable handling of amplified product
- All results should be interpreted by a health care professional in the context of patient medical history and clinical symptoms.
- This test cannot rule out diseases caused by other pathogens.
- Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.
- A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

## XV. Conditions of Authorization for the Laboratory

The Ezplex SARS-CoV-2 G Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas>

However, to assist clinical laboratories using the Ezplex SARS-CoV-2 G kit, the relevant Conditions of Authorization are listed below.

1. Authorized laboratories\* using the Ezplex SARS-CoV-2 G kit must include with result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
2. Authorized laboratories using the Ezplex SARS-CoV-2 G kit must use the Ezplex SARS-CoV-2 G kit as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the Ezplex SARS-CoV-2 G kit are not permitted.
3. Authorized laboratories that receive the Ezplex SARS-CoV-2 G kit must notify the relevant public health authorities of their intent to run the test prior to initiating testing.
4. Authorized laboratories using the Ezplex SARS-CoV-2 G kit must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
5. Authorized laboratories must collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: [CDRH-EUA-Reporting@fda.hhs.gov](mailto:CDRH-EUA-Reporting@fda.hhs.gov)) and SML Genetree (via email: [info@genetree.com](mailto:info@genetree.com) or [genetree@genetree.co.kr](mailto:genetree@genetree.co.kr)) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
6. All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the test in accordance with the authorized labeling.
7. SML Genetree, its authorized distributor(s) and authorized laboratories using the SARS-CoV-2 G kit must ensure that any records associated with this EUA are



maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

\* The letter of authorization refers to “Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests” as “authorized laboratories.”

## XVI. Performance Evaluation

### A. Analytical Sensitivity (Limit of Detection)

The limit of detection was evaluated for both upper and lower respiratory specimens. Negative clinical nasopharyngeal swabs collected in VTM and negative sputum matrix were used as the background matrices to prepare the samples. SARS-CoV-2 genomic RNA from Vircell (MBC137-R) was spiked into the negative clinical matrices at concentrations of 5, 2.5, 1.25, 0.625, and 0.3125 copies/uL. A total of 20 replicates per dilution were extracted using the QIAamp® DSP Virus Spin Kit and then tested on both the CFX96 Dx and ABI7500 thermocyclers. The preliminary LoD was then confirmed by testing 20 additional extraction replicates. The limit of detection was estimated as the concentration where the overall assay result yielded 95% positivity.

Target	CFX96		ABI 7500	
	Nasopharyngeal	Sputum	Nasopharyngeal	Sputum
RdRp	0.8 copies/uL	0.64 copies/uL	1 copies/uL	1 copies/uL
N	1 copies/uL	1 copies/uL	1 copies/uL	0.64 copies/uL
Overall LOD	1 copies/uL	1 copies/uL	1 copies/uL	1 copies/uL

### B. Analytical Sensitivity (Inclusivity)

An in silico analysis comparing the primer and probe sequences from the Ezplex SARS-CoV-2 G Kit against all SARS-CoV-2 sequences deposited in public databases (NCBI and GISAID) as of September 9, 2020 was conducted. The in silico analysis through reference sequences, generated the following data:

Target	Gene	Primer/Probe	Percent Identities
SARS-CoV-2 Virus	RdRp (NCBI, N=5000) (GISAID, N=5204)	Forward Primer	100 %
		Reverse Primer	100 %
		Probe	100 %
	N (NCBI, N=5000) (GISAID, N=5204)	Forward Primer	100 %
		Reverse Primer	100 %
		Probe	100 %

### C. Analytical Specificity

#### 1. Cross Reactivity

Nucleic Acid from a total of 24 species of microorganisms that are likely to be found in clinical respiratory specimens were tested with the Ezplex assay. Each species was tested in triplicate at the concentrations indicated in the table below. No cross reactivity was observed.

Microorganism	Concentration	Microorganism	Concentration
Adenovirus	1X10 <sup>4</sup> copies/uL	<i>Legionella pneumophila</i>	1X10 <sup>4</sup> copies/uL
Human metapneumovirus	1X10 <sup>6</sup> copies/uL	<i>Mycobacterium tuberculosis</i>	1X10 <sup>4</sup> copies/uL
Human parainfluenza virus 1	1X10 <sup>4</sup> copies/uL	<i>Streptococcus pneumoniae</i>	1X10 <sup>4</sup> copies/uL
Human parainfluenza virus 2	1X10 <sup>4</sup> copies/uL	<i>Bordetella pertussis</i>	1X10 <sup>4</sup> copies/uL
Human parainfluenza virus 3	1X10 <sup>4</sup> copies/uL	<i>Mycoplasma pneumoniae</i>	1X10 <sup>4</sup> copies/uL
Human parainfluenza virus 4	1X10 <sup>4</sup> copies/uL	<i>Candida albicans</i>	1X10 <sup>4</sup> copies/uL
Influenza A virus	1X10 <sup>4</sup> copies/uL	<i>Staphylococcus aureus</i>	1X10 <sup>4</sup> copies/uL
Enterovirus 71 type	1X10 <sup>4</sup> copies/uL	Human coronavirus OC43	1.41X10 <sup>6</sup> copies/uL
Human respiratory syncytial virus A	1X10 <sup>4</sup> copies/uL	Human coronavirus NL63	1X10 <sup>6</sup> copies/uL
Human respiratory syncytial virus B	1X10 <sup>4</sup> copies/uL	Severe acute respiratory syndrome	1X10 <sup>6</sup> copies/uL
Human rhinovirus	1X10 <sup>4</sup> copies/uL	Middle East respiratory syndrome	1X10 <sup>6</sup> copies/uL
<i>Chlamydomphila pneumoniae</i>	1X10 <sup>4</sup> copies/uL		
<i>Haemophilus influenzae</i>	1X10 <sup>4</sup> copies/uL		

#### 2. Cross Reactivity (in silico)

A total of 28 organisms were selected to assess the potential for cross reactivity with the primers/probes included in the Ezplex Assay. This analysis was conducted using the NCBI blast database. The percent homology with each primer and probe used in the Ezplex is displayed in the table below:

No.	Organisms	RdRP homology(%)			N homology(%)		
		F primer	Probe	R primer	F primer	Probe	R primer
1	Human coronavirus 229E*	<b>89%</b>	None	70%	58.8%	41.6%	50%
2	Human coronavirus OC43*	None	None	40%	<b>94%</b>	41.6%	50%

3	Human coronavirus HKU1*	<b>91.6%</b>	None	76.6%	52.9%	37.5%	60%
4	Human coronavirus NL63	33.3%	None	40%	52.9%	37.5%	60%
5	SARS-coronavirus **	75%	None	<b>93.3%</b>	<b>100%</b>	<b>83.3%</b>	<b>80%</b>
6	MERS-coronavirus*	60%	None	<b>90%</b>	58.8%	45.8%	60%
7	Adenovirus 71	37.5%	None	30%	52.9%	41.6%	50%
8	Human Metapneumovirus	45.8%	None	36.6%	58.8%	45.8%	55%
9	Parainfluenza virus 1	33.3%	None	33.3%	52.9%	37.5%	45%
10	Parainfluenza virus 2	None	None	None	None	None	None
11	Parainfluenza virus 3	None	None	None	None	None	None
12	Parainfluenza virus 4	50%	None	36.6%	64.7%	41.6%	50%
13	Influenza A	54.1%	None	43.3%	76.4%	62.5%	60%
14	Enterovirus group	50%	None	46.6%	70.5%	62.5%	70%
15	Respiratory syncytial virus	None	None	None	None	None	None
16	Rhinovirus	50%	None	46.6%	70.5%	62.5%	70%
17	<i>Chlamydia pneumoniae</i>	50%	None	43.3%	64.7%	54.1%	60%
18	<i>Haemophilus influenzae</i>	58.3%	None	43.3%	76.4%	54.1%	70%
19	<i>Legionella pneumophila</i> *	62.5%	None	50%	<b>82.3%</b>	54.1%	65%
20	<i>Mycobacterium tuberculosis</i>	50%	None	None	64.7%	62.5%	60%
21	<i>Streptococcus pneumoniae</i>	58.3%	None	43.3%	76.4%	54.1%	70%
22	<i>Streptococcus pyogenes</i>	54.1%	None	43.3%	76.4%	50%	65%
23	<i>Bordetella pertussis</i>	None	None	None	None	54.1%	70%
24	<i>Mycoplasma pneumoniae</i>	50%	None	43.3%	58.8%	45.8%	55%
25	<i>Pneumocystis jirovecii</i> (PJP)	62.5%	None	76.6%	64.7%	62.5%	65%
26	<i>Candida albicans</i> *	62.5%	None	53.3%	<b>88.2%</b>	54.1%	70%
27	<i>Pseudomonas aeruginosa</i>	62.5%	None	46.6%	76.4%	70.8%	65%
28	<i>Staphylococcus group</i> *	70.8%	None	50%	<b>82.3%</b>	79.1%	70%

1) For the organisms marked with \*, relatively high homology was observed (more than 80%) for one oligo in the primer/probe set. However, in order for an amplification product to be generated using the PCR mechanism, both of the primers and the probe must anneal to the target sites on the gene. Therefore, cross-reactivity with these organisms is not expected.

2) For the SARS-coronavirus organism marked with \*\*, more than 80% homology was found for all three oligos for the N gene. However, the interpretation algorithm of Ezplex states that only results where both the RdRp and N genes show amplification within 40 Ct are considered positive. Because >80% homology was not seen in all three

primer/probe oligos for the RdRp gene, false positive results are not expected to occur.

### 3. Interference

The effect of endogenous (Albumin (0.24g/mL), Hemoglobin (0.2g/mL), Bilirubin (0.05mg/mL) ) and exogenous (Mupirocin(20mg/mL), Tobramycin (80mg/mL), Zanamivir (250ug/mL)) interfering substances on assay performance was evaluated. Samples containing these substances were tested in triplicate with and without SARS-CoV-2 virus material at a concentration of ~3XLoD. No interference was observed as indicated by a less than 1 mean Ct differential between samples with and without interferent for both gene targets in all cases.

### D. In Silico Analysis

In silico analysis for all sequences of SARS-CoV-2, available from NCBI and GISAID databases, was conducted by mapping the individual primers and probes of the Ezplex assay. As of Sep 09, 2020, in silico analysis through GISAID (n =5204) and NCBI (n = 5000) sequences generated data as shown in the table below.

Gene	Primer(Probe)	Homology(%)	
		NCBI(N=5000)	GISAID(N=5204)
RdRp	Probe Primer	100	100
	Forward Primer	100	100
	Reverse Primer	100	100
N	Probe Primer	100	100
	Forward Primer	100	100
	Reverse Primer	100	100

### E. Clinical Evaluation

The purpose of this clinical evaluation was to assess the clinical performance of the Ezplex SARS-CoV-2 G Kit against an EUA-authorized comparator assay. In the clinical evaluation study, left-over archived specimens from symptomatic patients suspected of COVID-19 infection were tested. Specimens were previously subjected for SARS-CoV-2 testing and then stored at a clinical laboratory in South Korea prior to including in this study. A total of 30 positive and 30 negative clinical specimens confirmed by an EUA-authorized comparator Assay were tested with the investigational Ezplex assay. These 60 total specimens consisted of 15 positive and 15 negative upper respiratory (nasopharyngeal/oropharyngeal swabs collected in VTM) specimens and 15 positive and 15 negative sputum specimens. For this study, the specimens were extracted using the QIAamp® DSP Viral RNA mini Kit (Qiagen). Real-time RT-PCR was performed using the CFX96 Dx Real-time PCR Instrument (Bio-Rad). The results from testing of individual specimens are shown as below.

Upper Respiratory Specimens		Original Test of Record			Lower Respiratory Specimens		Original Test of Record		
		Positive	Negative	Total			Positive	Negative	Total
Ezplex	Positive	15	0	15	Ezplex	Positive	15	0	15
	Negative	0	15	15		Negative	0	15	15
	Total	15	15	30		Total	15	15	30
Positive agreement (95% CI)		100 % (78.2 ~ 100 %)			Positive agreement (95% CI)		100 % (78.2 ~ 100 %)		
Negative agreement (95% CI)		100 % (78.2 ~ 100 %)			Negative agreement (95% CI)		100 % (78.2 ~ 100 %)		

### XVII. FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded specimen testing was used to establish specificity and to confirm the LoD. The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded specimens and a standard testing protocol provided by the FDA. The extraction method and amplification instrument used were the QIAamp® DSP Viral RNA Mini Kit (62904) and the CFX96™ Dx Systems (1845097-IVD) respectively. The results are summarized in the Table below.

Table: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab in VTM	1,200 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL

N/A: Not applicable

ND: Not detected

## Appendix A: Additional Label

### For the ThermoFisher ABI 7500 Real-time PCR Instrument










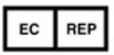

Please print and place this label on the front panel of the instrument. If the instrument includes labeling indicating “For Research Use Only”, please cover with the below “Emergency Use Only” labeling. The instrument should retain this labeling throughout the EUA use of the Ezplex SARS-CoV-2G Kit.

**Emergency Use Only  
This instrument is  
authorized for use**

## XVIII. References

1. **World Health Organization.** Q&A on coronaviruses (COVID-19). April 17, 2020. World Health Organization Web site <https://www.who.int/news-room/q-a-detail/q-a-coronaviruses>. Accessed October 8, 2020.
2. **Centers for Disease Control and Prevention.** <https://www.cdc.gov/coronavirus/2019-ncov/prevent-getting-sick/how-covid-spreads.html>. Accessed June 17, 2020.
3. **Centers for Disease Control and Prevention.** Coronavirus Disease 2019-(COVID-19) in the U.S. Updated October 7, 2020. Centers for Disease Control and Prevention website site <https://www.cdc.gov/coronavirus/2019-ncov/cases-in-us.html>. Accessed October 8, 2020.
4. **Centers for Disease Control and Prevention.** Coronavirus Disease 2019 Information for Travel. Page last reviewed August 21, 2020. Centers for Disease Control and Prevention website. <https://www.cdc.gov/coronavirus/2019-ncov/travelers/index.html>. Accessed October 8, 2020.
5. **Centers for Disease Control and Prevention.** Coronavirus Disease 2019-(COVID-19) Situation Summary. Update October 7, 2020. <https://www.cdc.gov/coronavirus/2019-ncov/summary.html>. Accessed October 8, 2020.
6. **Clinical & Laboratory Standards Institute.** Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections. CLSI website. <https://clsi.org/standards/products/microbiology/documents/m29>. Accessed October 8, 2020.

## XIX. Symbols and Information

Symbol	Meaning	Symbol	Meaning
	Storage Temperature		In-Vitro Diagnostic Medical Devices
	Expiration date		Product User Manual
	Catalogue Number		Manufacturer
	Lot Number		Keep away from sunlight (P+P)
	Contents sufficient for <n> tests		
 <b>Obelis S.A.</b> Bd. Général Wahis 53, B-1030 Brussels, Belgium	Authorized Representative in the European Community		For prescription use only

## XX. Technical and Customer Support

For Technical Support, please contact our Genetree Technical Support team. Before contacting Genetree Technical Support, collect the following information:

- Product name
  - Lot number
  - Software version
- Email for Technical Support: [technicalsupport@smlgenetree.com](mailto:technicalsupport@smlgenetree.com)
  - Email for Customer Support: [customersupport@smlgenetree.com](mailto:customersupport@smlgenetree.com)
  - Tel: +82-2-2057-7900
  - Fax: +82-70-7425-3950
  - Mailing address: 225 Baumoe-ro, Seocho-gu, Seoul, 06740 Republic of Korea
  - Website: <http://www.smlgenetree.com>

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400 Concar Drive, Suite 03-160  
San Mateo, CA 94402



# GENETREE VIEWER SOFTWARE (1.13.777)

## For Use with Ezplex SARS-CoV-2 G Kit

For Emergency Use Authorization (EUA) only.

For *in vitro* diagnostic use only.

For prescription use only

## Operators Manual



**Manufacturer:**

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FAX : +82-70-7425-3950

Website : <http://www.genetree.co.kr>

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## 1. Intended Use

Genetree Viewer is software used for the analysis of the qualitative detection of SARS-CoV-2 Virus using the Ezplex SARS-CoV-2 G Kit, a Reverse Transcription Real-time Polymerase Chain Reaction test that is able to detect SARS-CoV-2 Virus by extracting ribonucleic acid (RNA) from nasopharyngeal/oropharyngeal swabs and sputum specimens from patients suspected of having the COVID-19 infection.

## 2. Product Description and Instructions for Use

### 2.1 System Requirements

- 1) Microprocessor: Intel(R) i3 3.5 GHz or above
- 2) Memory: 4GB or larger
- 3) Microsoft Windows 7 or above
- 4) More than 1 USB port on computer

### 2.2 Software Download

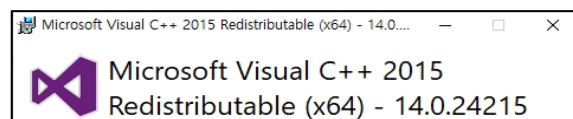
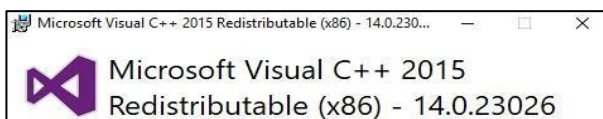
1. Insert the Genetree Viewer Software usb into the USB port of your computer. Download the Genetree Viewer software.
2. When the download is completed, extract the downloaded zip file to an appropriate folder.

### 2.3 Software Installation

1. Before installing Genetree Viewer analysis software, both of the '*Microsoft Visual C++ 2015 Redistributable(x86) Package*' and the '*Microsoft Visual C++ 2015 Redistributable(x64) Package*' must be installed in advance.

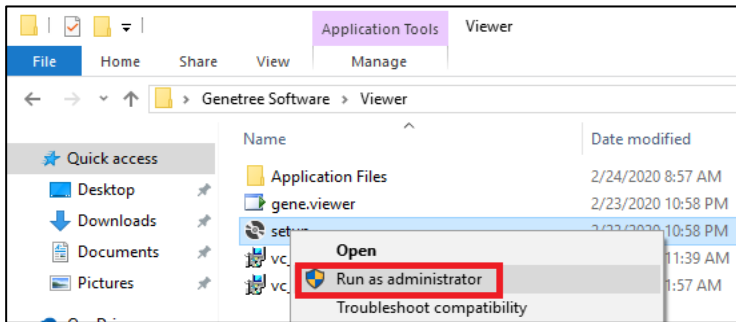
**NOTE: Both packages are contained in the Genetree Viewer installation zip file.**

**NOTE: The Microsoft Visual C++ 2015 Redistributable(x86) is saved as "vc\_redist.x86" and The Microsoft Visual C++ 2015 Redistributable(x64) is saved as "vc\_redist.x64"**

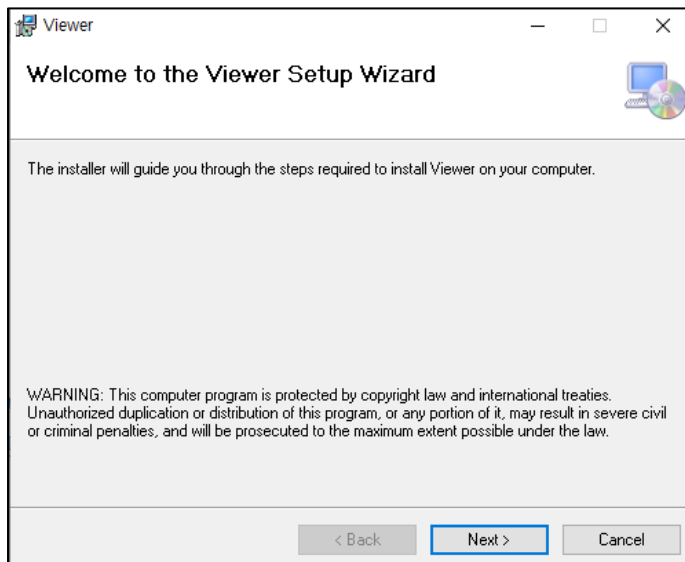


2. After the pre-installation step, click on **'Run as Administrator'** in the **'Setup.exe'** file in the installation folder of **'Genetree Viewer'**.

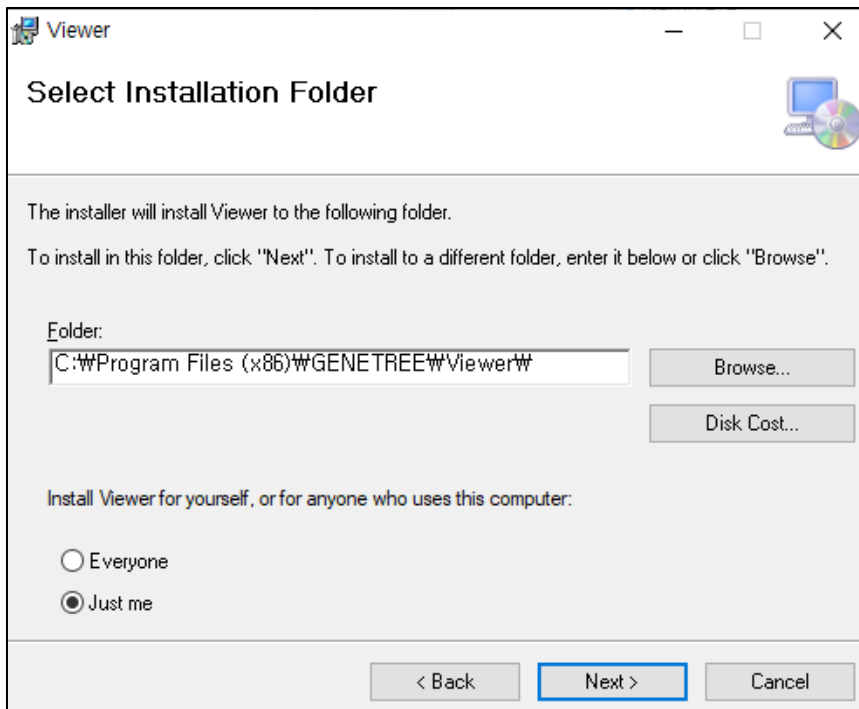
**NOTE: If the installation is not possible due to the presence of antivirus software, the installation can be performed by temporarily stopping or not running the antivirus software and proceeding with the installation.**



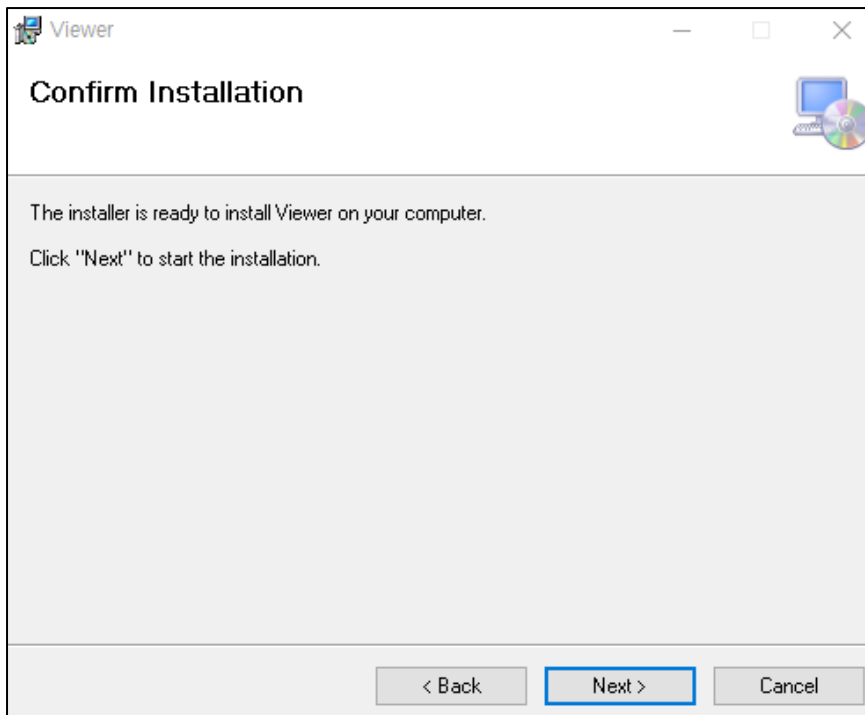
3. If **Run as Administrator**, GENETREE VIEWER Setup Wizard will pop up and guide the user through the steps required to install Viewer on your computer. Please, Click on **'Next'**.



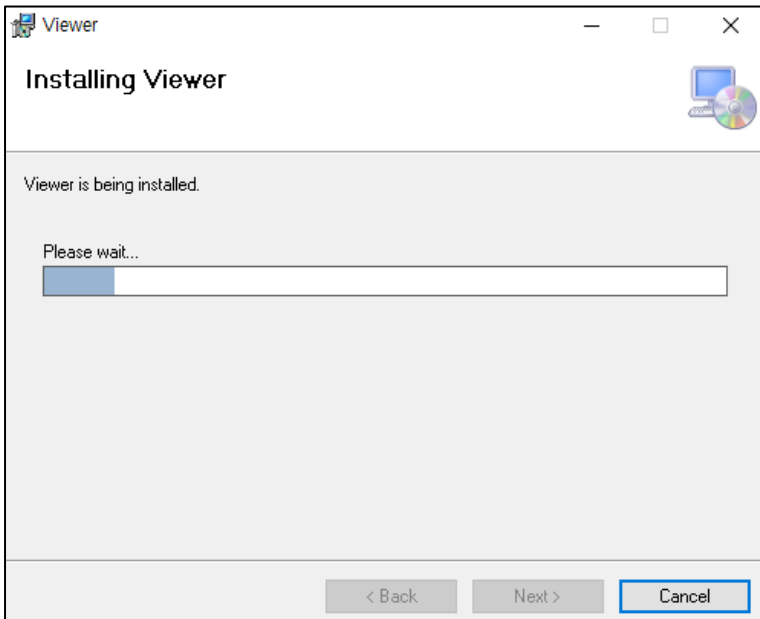
4. Specify the install directory and check whether GENETREE VIEWER is installed for yourself or for anyone who uses this computer. And Click on **'Next'**



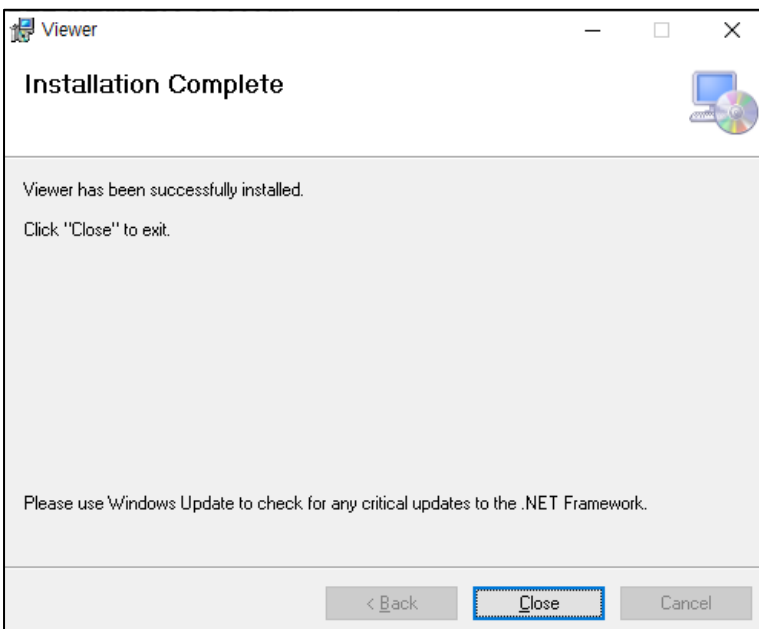
5. Click on **'Next'** to start installation.



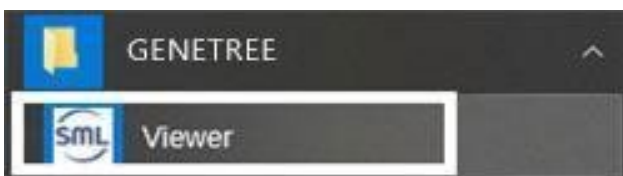
6. Wait for installation completion



7. After installation Complete, Click on 'Close'.

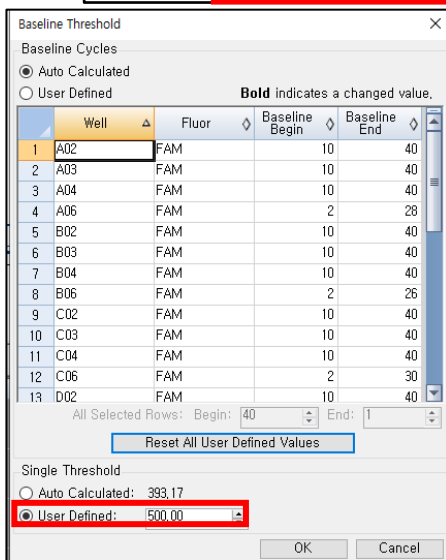
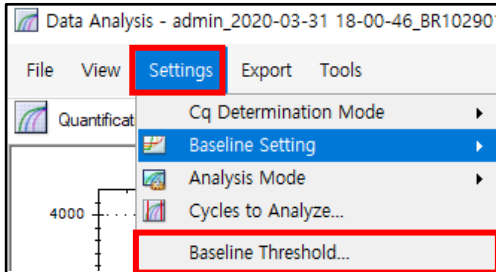


8. If the installation is completed, the run file of analysis software can be found in the 'Start menu' as shown below.

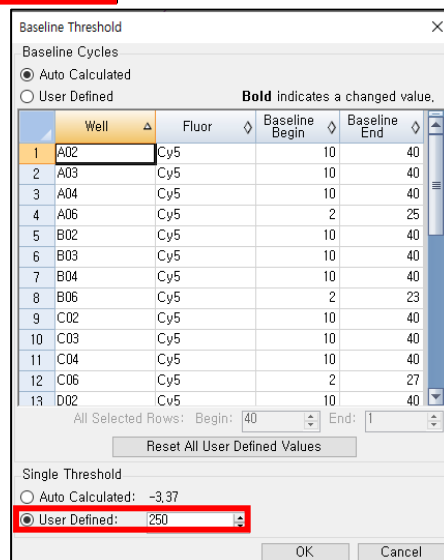


## 2.4 Software Analysis (Bio-Rad CFX96 Dx Real-time PCR Instrument)

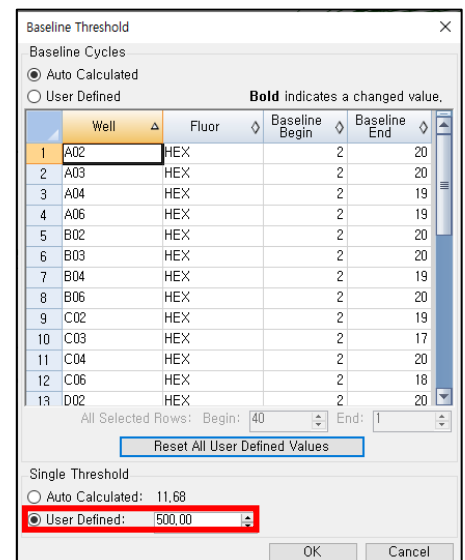
1. Check that PCR is finished and if the threshold is applied as in 'Ezplex SARS-CoV-2 Kit Insert' in the CFX Manager software.



<FAM>



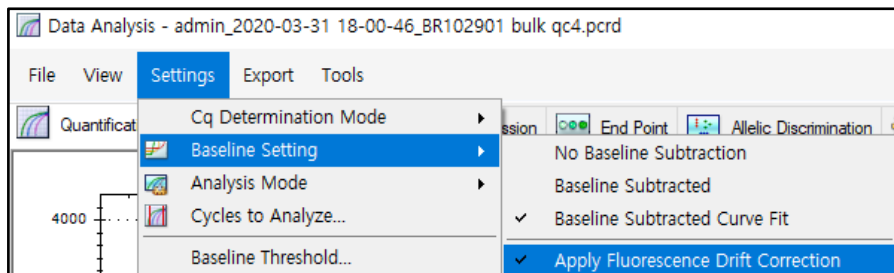
<CY5>



<HEX>

2. Click to enable the apply fluorescence drift correction in the CFX Manager software.

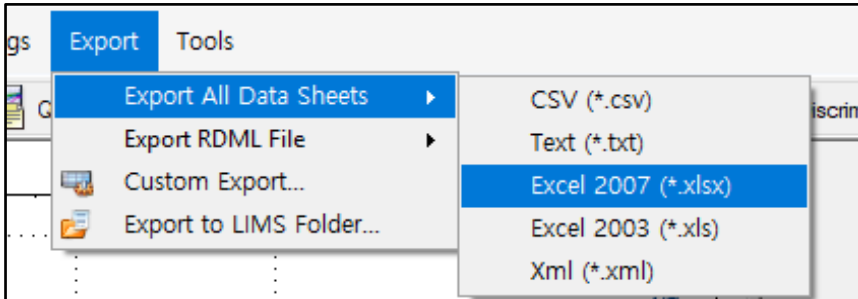
**Note: This configuring step is only needed for the CFX Manager software 3.1 version. In the CFX manager 1.6, steps '1' and '2' can be skipped.**



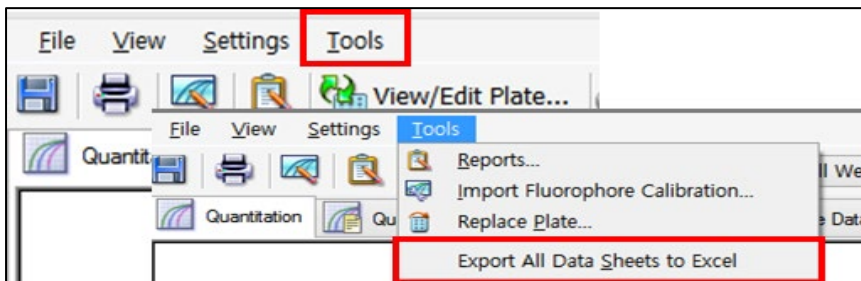
- Click **'Export All Data Sheets'** from CFX Manager software's **'Tool'** menu to convert the test data into an excel spreadsheet.

**Note: Select Excel 2003 or Excel 2007 according to the Excel specifications.**

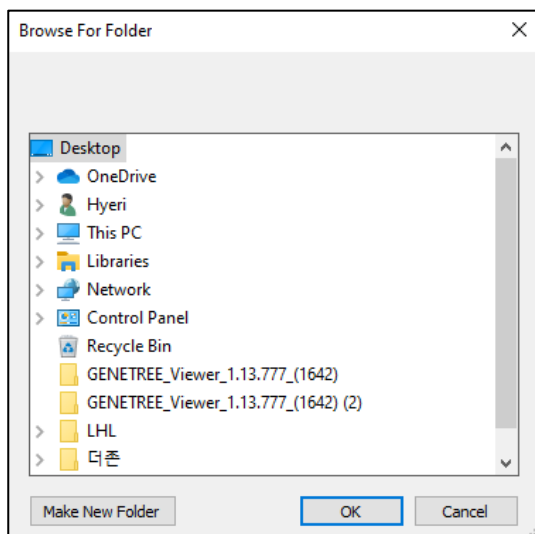
<CFX Manager software: 3.1 version>



<CFX Manager software: 1.6 version>

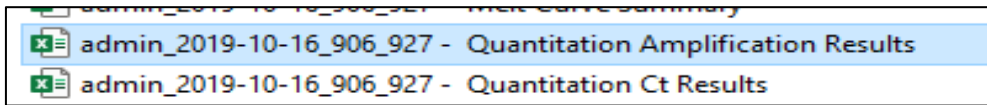


- Specify a folder to save the file if you want, make New Folder and save the file in it

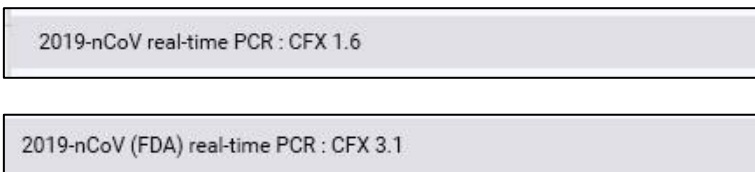




- Run the analysis software (Genetree Viewer); select **'Open'** on the upper left to navigate the folder where the converted excel file is saved, and open the file with name that ends with 'Quantitation Amplification Results.'

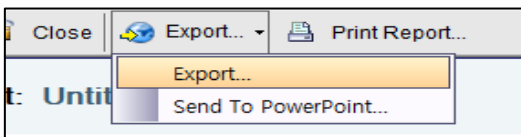


- Click **'Please select a kit'** menu at the top of the screen and select the appropriate item for the tested batch of specimens (2019-nCoV real-time PCR: CFX1.6 and 3.1).



### 2.5 Software Analysis (ABI 7500 Real-time PCR Instrument)

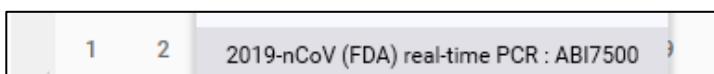
- Run the 7500 software, click the **'Export'** in the export menu.



- When the Export Properties window appears, select the 'Result' and 'Amplification' data in the '1. Select data to export'. Select data to export' checkbox, specify the Excel file name in **'ExportFile Name'**, and proceed the export of data files.



- Run the 'Genetree Viewer' software, and load the exported data files via **'Open'** menu.
- When the files are successfully loaded, click the **'Please select a kit'** and select the appropriate test item as below.



- As shown in the image below, results for each well can be checked according to the selected Kit component.

Note: Refer to the table below for description of the results.

3.1 General Description of the Software and Example Screens

No.	Description
①	Positive/Negative results by well are indicated in '+', '-' respectively. Invalid results by well are indicated in ' ' respectively. Inconclusive results by well are indicated in '?' respectively.
②	Ct and fluorescent values of the results for each well are plotted on a graph.
③	Ct values of the results for each well are indicated numerically and qualitative results are printed.
④	Analysis results are converted into an excel spreadsheet.

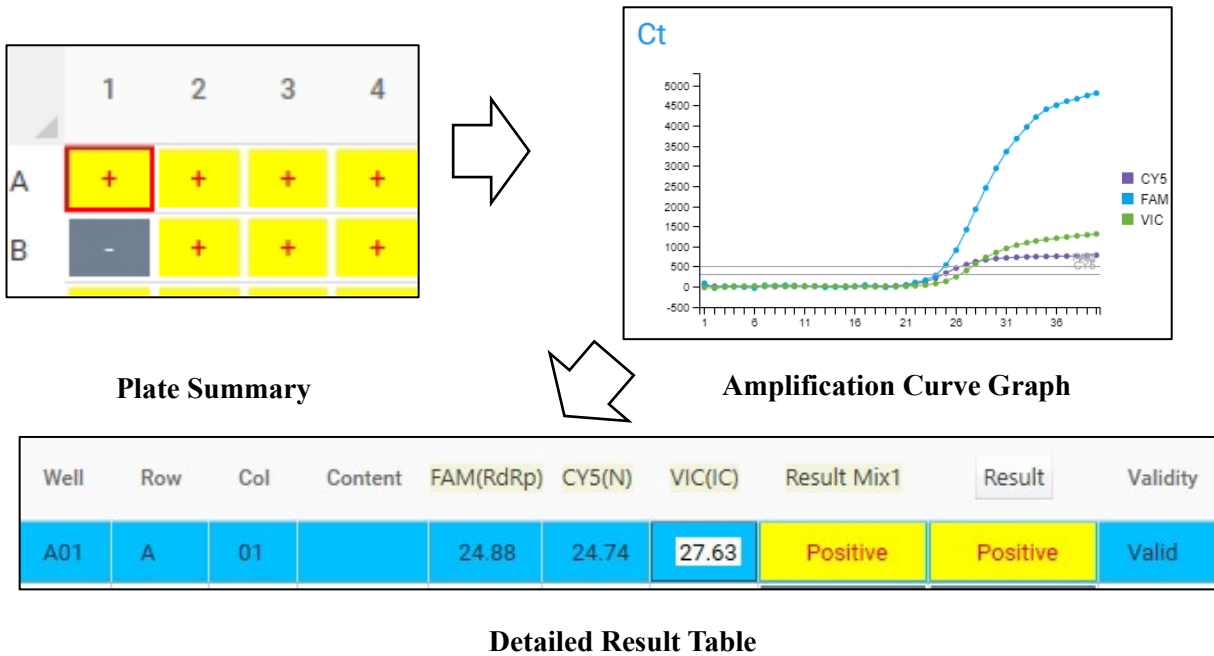
The screenshot shows the GENETREE VIEWER 1.13.777 interface. At the top, there are menu options like 'Open', 'Threshold', 'Print', and 'ExportToExcel'. Below this is a 96-well plate visualization (rows A-H, columns 1-11) with colored cells representing results: '+' (yellow), '-' (grey), '?' (white), and '|' (black). A red box labeled '1' highlights a well with '+'. To the right is a Ct graph showing fluorescence intensity vs. cycle number for three channels: CYS (blue), FAM (green), and VIC (red). A red box labeled '2' highlights the FAM curve. Below the graph is a data table with columns: Sample ID, Sample, Well, Row, Col, Content, FAM(RdRp), CYS(N), VIC(IC), Result Mix1, Result, Validity, Comment. A red box labeled '3' highlights the 'Result' column. At the bottom is an exported Excel spreadsheet with columns: No., Sample ID, Sample, Well, Content, RdRp, N, IC, Result, Validity, Comment. A red box labeled '4' highlights the 'Result' column. A red arrow points from the 'ExportToExcel' button to the spreadsheet.

Sample ID	Sample	Well	Row	Col	Content	FAM(RdRp)	CYS(N)	VIC(IC)	Result Mix1	Result	Validity	Comment
		B02	B	02		36.06	36.97	27.75	Positive	Positive	Valid	
		C02	C	02		36.96	38.28	27.68	Positive	Positive	Valid	
		D02	D	02		39.14	37.61	27.62	Positive	Positive	Valid	
		E02	E	02		39.04	N/A	27.51	Inconclusive	Inconclusive	Valid	
		F02	F	02		N/A	N/A	27.50	Negative	Negative	Valid	
		G02	G	02		N/A	N/A	27.41	Negative	Negative	Valid	
		H02	H	02		N/A	N/A	N/A	Invalid	Invalid	Invalid	Retest after re-extraction
		A03	A	03		36.12	37.22	27.88	Positive	Positive	Valid	
		B03	B	03		35.71	36.67	27.69	Positive	Positive	Valid	

No.	Sample ID	Sample	Well	Content	RdRp	N	IC	Result	Validity	Comment
10			B02		36,06	36,97	27,75	Positive	Valid	
11			C02		36,96	38,28	27,68	Positive	Valid	
12			D02		39,14	37,61	27,62	Positive	Valid	
13			E02		39,04	N/A	27,51	Inconclusive	Valid	
14			F02		N/A	N/A	27,50	Negative	Valid	
15			G02		N/A	N/A	27,41	Negative	Valid	
16			H02		N/A	N/A	N/A	Invalid	Invalid	Retest after re-extraction
17			A03		36,12	37,22	27,88	Positive	Valid	
18			B03		35,71	36,67	27,69	Positive	Valid	

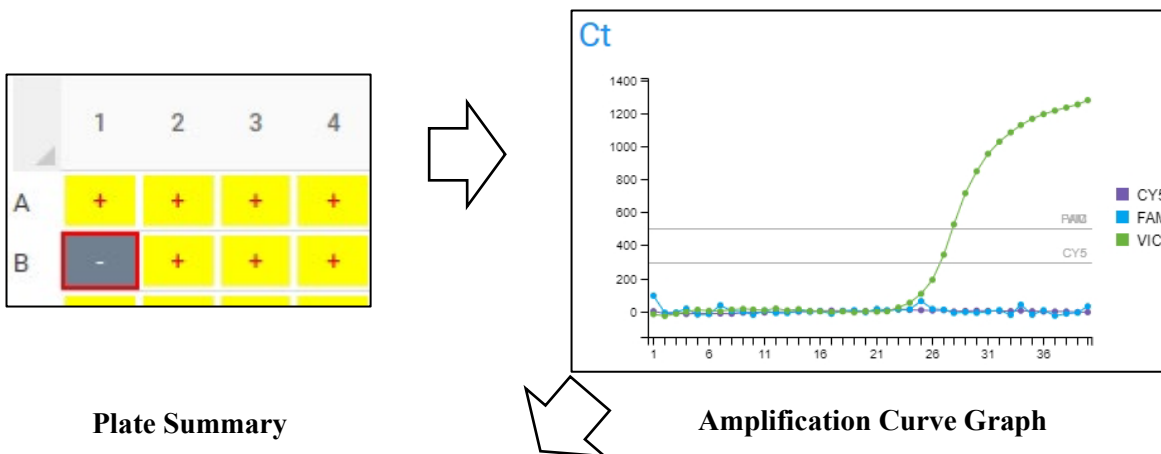
### 3.2 Positive Sample Example

The '+' mark indicates a positive result in the plate summary. All the amplification curves appear above each threshold line in the amplification curve graph. The detailed result table displays each Ct value, positive result, and its validity for the positive sample.



### 3.3 Negative Sample Example

The '-' mark indicates a negative result in the plate summary. The amplification curves for RdRp and N genes do not appear above each threshold line in the amplification curve graph (Only HEX or VIC appear). The detailed result table displays each Ct value as N/A, negative result, and its validity for the negative sample.



Well	Row	Col	Content	FAM(RdRp)	CY5(N)	VIC(IC)	Result Mix1	Result	Validity
B01	B	01		N/A	N/A	27.86	Negative	Negative	Valid

Detailed Result Table

### 3.4 Inconclusive Sample Example

The '?' mark indicates an inconclusive result in the plate summary. Just one of the amplification curves for RdRp or N gene appear above each threshold line in the amplification curve graph (HEX or VIC also appears). The detailed result table displays each Ct value, inconclusive result, and its validity.

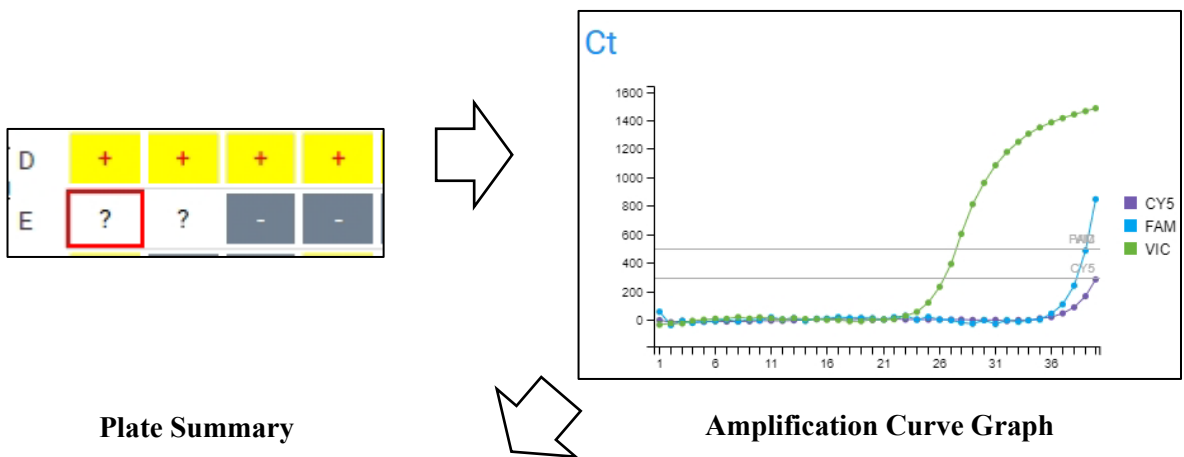


Plate Summary

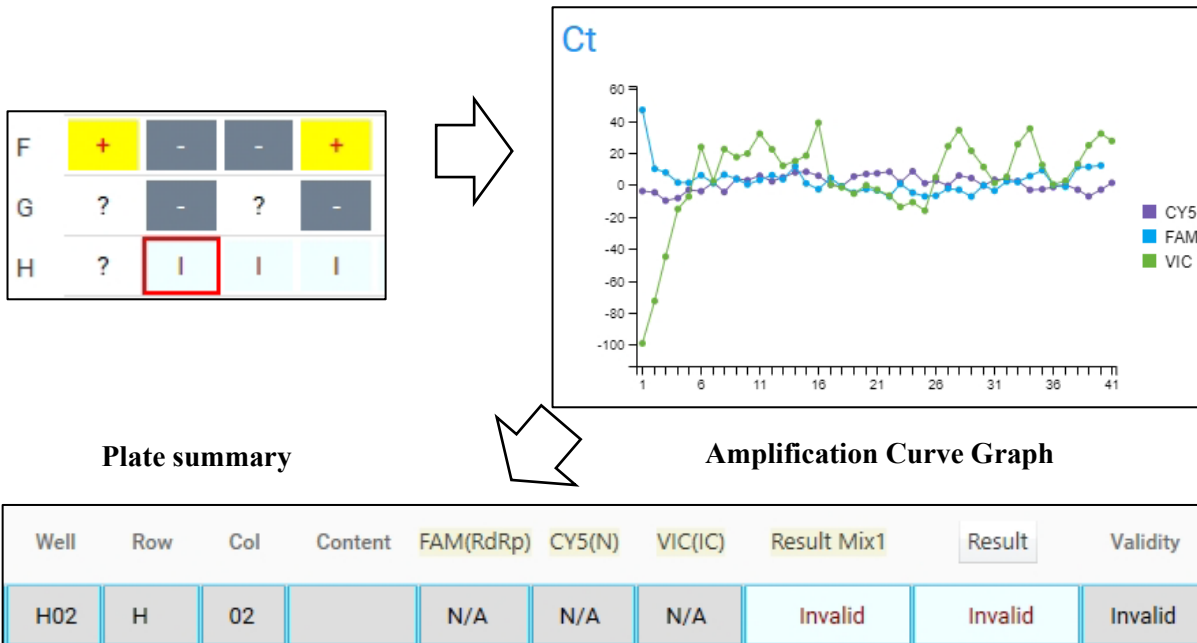
Amplification Curve Graph

Well	Row	Col	Content	FAM(RdRp)	CY5(N)	VIC(IC)	Result Mix1	Result	Validity
E01	E	01		N/A	37.60	27.76	Inconclusive	Inconclusive	Valid

Detailed Result Table

### 3.5 Invalid Sample Example

The 'I' mark indicates an invalid result in the plate summary. The amplification curve for IC (HEX or VIC) does not appear above its threshold line in the amplification curve graph (RdRp and N gene may or may not appear). The detailed result table displays each Ct value(N/A in IC), invalid result, and its invalidity.



Detailed Result Table






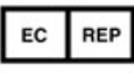
### 3. Troubleshooting

The following table provides troubleshooting information and instructions for the Operator to follow to attempt to remedy abnormal situations that may arise with the Genetree Viewer software. Contact Technical Support if these or any other situations arise regarding the instrument or tests that cause concern.

No.	Relating Task	Symptom	Possible cause	Possible solution
1	Data File Open	Error Message: There is not a matched instrument for the input file.	Wrong format file selected and opened.	Select and reopen the appropriate data file exported from the device software (See 2.4 and 2.5).
2	Selecting a Kit	Cannot see correct test kit name.	Wrong data file opened which has different fluorescence channels with test assay.	Make sure that the test result was saved correctly and open it again.
3	Selecting a Kit	The question mark '?' is on the	The test kit is yet to be	Select an appropriate test kit.

		whole plate well.	selected.	
4	Analysis	Displayed positive result of different organisms, not SARS-CoV-2 Virus.	Wrong data file opened which has similar fluorescence channel with tested assay.	Make sure that the test result was saved correctly and open it again.
5	Analysis	Cannot see Ct graph on positive result	The opened data file might be corrupted.	Re-export the test data from the device software.
6	Analysis	Displays "Negative" on the samples even though amplification occurred.	The threshold might not be adjusted in accordance with the Test kit instructions for use.	Readjust the threshold referring to the Test kit instructions for use.

#### 4. Symbols

Symbol	Meaning	Symbol	Meaning
	Catalogue Number		In-Vitro Diagnostic Medical Devices
	Manufacturer		Product User Manual
	European Conformity		Authorized Representative in the European Community

#### 5. Authorized European Representative



**Obelis S.A**

Address:

Bd. Général Wahis 53, B-1030 Brussels, Belgium

#### 6. Technical Support

For Technical Support, please contact our dedicated technical support team on:

For Technical Support, please contact our Genetree Technical Support team. Before contacting Genetree Technical Support collect the following information:

- Product name
- Lot number
- Error message (if any)

- Software version
- Email for Technical Support: [info@smlgenetree.com](mailto:info@smlgenetree.com)
- Email for Customer Support : [info@smlgenetree.com](mailto:info@smlgenetree.com)
- Tel: +82-2-2057-7900
- Fax: +82-70-7425-3950
- Mailing address: 6F, Hanmaeum Bldg, 225 Baumoe-ro, Seocho-gu, Seoul, 06740
- Republic of Korea
- Website: <http://www.smlgenetree.com>

### Warnings – When Used with the Ezplex SARS-CoV-2 G Kit:

- For *in vitro* diagnostic use. For use under an Emergency Use Authorization (EUA) only.
- This product has not been FDA cleared or approved; but has been authorized by the FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. §360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

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