Instructions for Use

(Ezplex® 2019-nCoV Real-time PCR kit)

1. Product Name

Ezplex® 2019-nCoV Real-time PCR Kit

2. Manufacturer (Name and Address)

- Name: SML Genetree Co., Ltd. / 82-2-2057-7900
- Address: 6F, Hanmaeum Bldg., 225 Baumoe-ro, Seocho-gu, Seoul, South Korea.

3. Intended Use

The product is an in-vitro diagnostic medical device that is used for qualitative detection of SARS-CoV-2 Virus by extracting ribonucleic acid (RNA) from Nasopharyngeal and Oropharyngeal swab specimens from patients suspected of having the COVID-19 infection and by using the Real-time Reverse Transcription Polymerase Chain Reaction.

4. Instructions for use

4.1. Specimen Preparation and Storage
A. Nasopharyngeal and Oropharyngeal swab specimens shall be used for the test.
B. It is recommended that swab specimens shall be used immediately after collection. However, the specimens can be stored maximum 4 days at 2-8°C in a fridge or maximum 2 months at –20°C in a freezer if immediate use is not achievable.
C. Specimens shall be divided into amounts required for one testing and stored at –20°C in a freezer so as to avoid from thawing repeatedly.
D. Specimens that are no longer needed shall be put in a container for liquids and disposed as liquid medical waste.
E. Specimen collection
- Specimens shall be collected in a dedicated container which shall be sealed to prevent leakage.
- Adequate protective gears such as gloves and gowns shall be used to handle the specimens.
- Protective glasses, masks, or aprons shall be worn if protection is required against specimen splatter.

4.2. Pre-test Preparations

A. Reagents shall be stored at –20°C and shall avoid from repeated freezing and thawing.
B. Reagents shall be used after completely thawed.
C. Since RNA can be degraded from the positive control, it is recommended that the reagents shall be divided into amounts required for 1-2 tests and stored in a freezer.
D. Equipment required for testing: **CFX96 Real-time PCR (Bio-Rad)**

4.3. Test Procedure

A. Specimen Pretreatment
While it is possible to use various ways and kits adopted in laboratories to extract RNA and apply on this product, it is recommended that QIAamp DSP Virus Spin Kit (Qiagen GmbH) shall be used for RNA extraction and users shall follow the protocol included in the Kit Handbook. After being extracted, RNA shall be stored at –20 ± 2°C in a freezer and shall be divided into amounts required for 1-2 tests since RNA can be degraded.

B. Real-time PCR Amplification
1) Making reagent master mix solution

① Refer to the tables below and make PCR master mix solution according the number of samples to be tested (See Table 1, 2).

Table 1. In case a separate IC is included in specimen extraction (unit: uL)

Component	Capacity
RQ Mixture	10
nCoVP+P	5
Total	15

Table 2. In case a separate IC is not included in specimen extraction (unit: uL)

Component	Capacity
RQ Mixture	10
nCoVP+P	5
IC	0.1
Total	15.1

② Divide 15㎕ of PCR mater mix solution in PCR tubes, add 5㎕ of the RNA specimen in each tube, and mix them well.

③ Both positive control and negative control shall be tested for accuracy.

2) Set up the device with below conditions.

Step	Temperature / Time	Cycle
Hold	25 ºC / 2 min	1 Cycle
	50 ºC / 30 min	
	95 ºC / 5 min	
Cycle	95 ºC / 15 sec	40 cycles
	60 ºC / 45 sec	

4.4. Results

4.4.1 Fluorescent thresholds for detection targets were set **'500' for FAM, HEX, '150' for Cy5**, and, **'100' for Quasar705,** after which Ct value was checked to decide the results according to the below table.

FAM (RdRp)	HEX (E)	Cy5 (N)	Quasar 705(IC)	Result*	Remark
<40	<40	<40	Any	**Positive**	
<40	≥40 or Neg	<40	Any	**Inconclusive***	
<40	<40	≥40 or Neg	Any	**Inconclusive***	
≥40 or Neg	<40	<40	Any	**Inconclusive***	
<40	≥40 or Neg	≥40 or Neg	Any	**Negative****	
≥40 or Neg	<40	≥40 or Neg	Any	**Negative****	
≥40 or Neg	≥40 or Neg	<40	Any	**Negative****	
≥40 or Neg	≥40 or Neg	≥40 or Neg	<38	Negative	
≥40 or Neg	≥40 or Neg	≥40 or Neg	≥38 or Neg	Invalid	Retest after re-extraction

*** The result is judged as Positive only when it is detected all of RdRp, E and N gene. Further confirmatory test shall be necessary if the result is judged as "Inconclusive".**
**** If single gene is detected alone, regardless of the gene, the result is judged as Negative.**

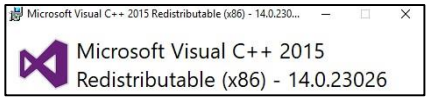
4.4.2 Positive&Negative control range
Due to PCR instruments managing in all different conditions, the individual fluorsence thresholds are changeable if controls are deviated from measured ranges as below table.

Control	RdRp Ct (FAM)	E Ct (HEX)	N Ct (Cy5)	IC Ct (Quasar 705)
PC	24.5 ~ 26.5	25.0 ~ 27.0	22.0 ~ 24.0	Any
NC	Neg	Neg	Neg	26.5 ~ 28.5

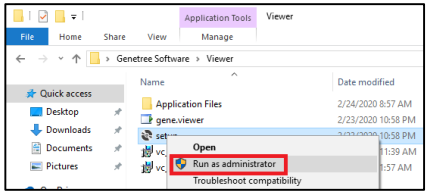
4.4.3 Software analysis(Genetree Viewer)

4.4.3.1 Software installation

1) Before installing analysis software, the 'Microsoft Visual C++ 2015 Redistributable(x86)' shall be installed in advance.



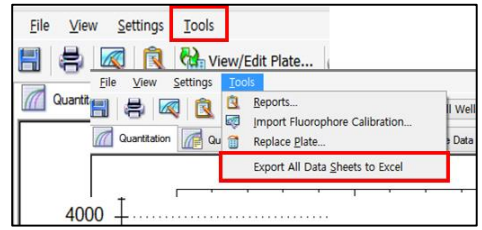
2). After pre-installation step, click on 'Run as administrator' of file 'Setup.exe' in the installation folder of 'Genetree Viewer'.
NOTE : Please contact 'genetree@genetree.co.kr' to acquire 'Genetree viewer' software.



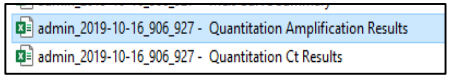
3) If the installation is completed, the run file of analysis software can be found in the 'Start menu' as below.

4.4.3.2 Software Analysis

1) Check that PCR is finished and click 'Export All Data Sheets to Excel' from CFX96 Manager software's 'Tool' menu to convert the test data into an excel spreadsheet (Create a folder and save the file in it).
NOTE : The Genetree Viewer software is only compatible with CFX96 Manager version 1.6. If other version is used for PCR running, manual analysis shall be performed referring to '4.4.1'.

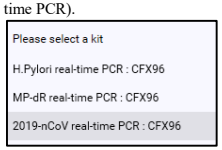


2) Run the analysis software (Genetree Viewer), press 'Open' on the upper left to navigate the folder where the converted excel file is saved, and open the file with name that ends with 'Quantitation Amplification Results.'



SML GENETREE, INC. 6F, Baumoe-ro 225, Seocho-gu, Seoul, Korea
Email : genetree@genetree.co.kr / Tel: 02-2057-7900 / Fax: 070-7425-3950

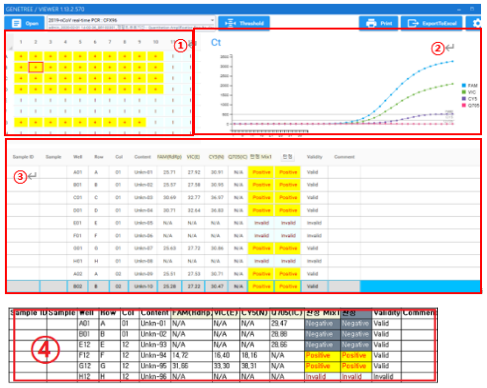
3) Click 'Please select a kit' menu at the top of the screen and select an appropriate item for the tested panel as in the below figure (2019-nCoV real-time PCR).



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

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

No.	Description
①	Positive/Negative 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.



5. Warnings and Precautions

1) This product is intended for diagnostic use, and shall be used by clinical expert such as clinical pathologist and medical technologist.
2) Insufficient test results, such as inconclusive result, through this product shall be confirmed together with additional diagnostic measures.
3) All product components shall be taken out just before use and shall be stored in a freezer (below –20°C) immediately after use they are exposed as little as possible to the ambient temperature.
4) Beware of carry-over contamination since the Real-time PCR has a high sensitivity.
5) Repeated freezing and thawing of reagent and specimen shall be avoided because they may affect the test sensitivity.
6) Beware of microbe contamination when dividing the reagent and it is recommended to use a sterilized disposable filter tip.
7) Beware not to touch the reagent container cap or the inner side of PCR tube cap with your hands.
8) It is prohibited to mix the products from different Lots even in case of the same product's reagent.
9) Do not use the product if the use authorization is expired.
10) Tests shall be performed in accordance with the Guideline for Laboratory Biosafety and the Laboratory Safe Management Manual.
11) While handling the specimen, beware of infection through skin or inhalation. In case of human exposure, the part shall be immediately cleansed with running tab water and medical attention shall be sought immediately for symptoms including high fever and rashes.
12) Tests shall be performed in accordance with the Guideline for Laboratory Biosafety and the Laboratory Safe Management Manual, and all spaces shall be thoroughly sterilized using 70% Ethanol or 0.5% sodium hypochlorite.

6. Performance

<table>
<tr><th>No</th><th>Test Name</th><th>Test method</th></tr>
<tr><td>1</td><td>Analytical Sensitivity (Limit of Detection)</td><td>Using synthesized RNA of RdRp, E and N gene, it was serially spiked in both of nasopharyngeal and oropharyngeal swab specimens, and RNA were extracted from those prepared specimens. The test was performed ten times on every diluted concentrations and the limit of detection is calculated as below using probit analysis of 95% positive rate.
<table>
<tr><th>Target</th><th>Limit of detections(copies/㎕)</th></tr>
<tr><td>RdRp</td><td>1.842</td></tr>
<tr><td>E</td><td>0.467</td></tr>
<tr><td>N</td><td>1.842</td></tr>
</table></td></tr>
<tr><td>2</td><td>Analytical Specificity (Cross reactivity)</td><td>The fifteen species of DNA and RNA materials, which are expected of cross reactivity, are chosen for the test and the test repeated three times on every chosen species. As a result of test, there were no cross reactivity as those were observed as all negative result.
<table>
<tr><th>No.</th><th>Marterials</th></tr>
<tr><td>1</td><td>Influenza A H3</td></tr>
<tr><td>2</td><td>Influenza B</td></tr>
<tr><td>3</td><td>Respiratory Syncytial Virus A</td></tr>
<tr><td>4</td><td>Respiratory Syncytial Virus B</td></tr>
<tr><td>5</td><td>Parainfluenza virus 1</td></tr>
<tr><td>6</td><td>Parainfluenza virus 2</td></tr>
<tr><td>7</td><td>Parainfluenza virus 3</td></tr>
<tr><td>8</td><td>Coronavirus OC43</td></tr>
<tr><td>9</td><td>Coronavirus 229E</td></tr>
<tr><td>10</td><td>Coronavirus NL63</td></tr>
<tr><td>11</td><td>Enterovirus 71</td></tr>
<tr><td>12</td><td>Adenovirus</td></tr>
<tr><td>13</td><td>Rhinovirus</td></tr>
<tr><td>14</td><td>Chlamydophila pneumoniae</td></tr>
<tr><td>15</td><td>Legionella pneumophila</td></tr>
</table></td></tr>
<tr><td>3</td><td>Analytical Specificity (Interference)</td><td>The interference materials were prepared with Albumin (0.24g/mL), Hemoglobin (0.2g/mL) and Billirubin (0.05mg/mL), those were tested three times with and without positive materials of RdrRp, E and N genes diluted in 2 copies/uL, which are the lowest detection concentration in LoD test. As a result of the test, there were no interference by observing the coefficient of variation(CV) value which were less than 5% in all cases.</td></tr>
<tr><td>4</td><td>Precision (Reproducibility, Repeatability)</td><td>The test materials were prepared as positive and negative control, positive divided into high and mid-concentration of the synthesized RNA materials, and D.D.W was used as negative control. Those materials were tested totally ten times each, repeating five times daily for two days. As a result of the test, the high precision has been confirmed by observing the coefficient of variation(CV) value which were less than 5% in all cases.</td></tr>
<tr><td>5</td><td>Clinical Performance</td><td>The clinical performances of 53 oropharyngeal and nasopharyngeal swab samples, in which confirmed through a certified another IVD reagent, were collected and performed as below.
<table>
<tr><th>Specimens</th><th>Clinical sensitivity (95 % CL)</th><th>Clinical specificity (95 % CL)</th></tr>
<tr><td>Nasoparyngeal</td><td>100 % (47.8 ~100%)</td><td>100 % (84.6 ~100%)</td></tr>
<tr><td>Oropharyngeal</td><td>100 % (47.8 ~100%)</td><td>100 % (83.9 ~100%)</td></tr>
</table></td></tr>
</table>

7. Storage

A. Storage

Reagent Name	Before/After opening the container	Storage Condition	Shelf life
RQ Mixture	Before opening	-20°C	64 days from date of manufacture
nCoV P+P	Before opening	-20°C	
Positive control	Before opening	-20°C	
Negative control	Before opening	-20°C	
Internal control	Before opening	-20°C	

B. Storage and transport conditions

1) Products that are packaged shall be stored in a storage freezer.
2) To transport the products to a client, the products shall be put in a cooler with dry ice so that the products are not exposed to the ambient temperature.
3) When sending the products via a courier service, product boxes shall be wrapped with bubble wraps before putting in a cooler, ices packs shall be stacked on the products, and then dry ice shall be filled to cover more than 1/3 of the entire box so that temperature change during delivery is minimized.

8. Packing Unit

* 100 Test Kits

No	Component	Presentation
1	RQ Mixture	1 vial, 1000 uL
2	nCoV P+P	1 vial, 500 uL
3	Positive control	1 vial, 50 uL
4	Negative control	1 vial, 50 uL
5	Internal control	1 vial, 20 uL

* 200 Test Kits

No	Component	Presentation
1	RQ Mixture	2 vials, 1000 uL
2	nCoV P+P	2 vials, 500 uL
3	Positive control	2 vials, 50 uL
4	Negative control	2 vials, 50 uL
5	Internal control	2 vials , 20 uL

SML GENETREE, INC. 6F, Baumoe-ro 225, Seocho-gu, Seoul, Korea
Email : genetree@genetree.co.kr / Tel: 02-2057-7900 / Fax: 070-7425-3950