Ezplex®
Viral Respiratory Real-time PCR Kit

Instructions for Use (IFU)

IVD

Approved by MFDS (Ministry of Food and Drug Safety of South Korea)

100 Tests

Manufacturer:

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I. Intended Use

The Ezplex® Viral Respiratory Real-time PCR Kit performed on the Bio-Rad CFX96 Dx Real-time PCR instrument, is a multiplex *in-vitro* diagnostic test intended for the simultaneous qualitative detection and differentiation of Influenza A, Influenza H1N1, Influenza H3N2, Pandemic H1N1/09 virus, Influenza B, Respiratory syncytial virus A, Respiratory syncytial virus B, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Adenovirus, Metapneumovirus, Enterovirus, Bocavirus, Rhinovirus A/B/C, Coronavirus 229E, Coronavirus OC43, and Coronavirus NL63 by extracting nucleic acid (RNA) from nasopharyngeal swabs from patients suspected of having respiratory infection.

Nucleic acids from the respiratory viral organisms identified by this test are generally detectable in nasopharyngeal swabs (NP) during the acute phase of infection. Positive results from individuals exhibiting signs and/or symptoms of respiratory infection are indicative of the presence of the identified microorganism(s); clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results are indicative of the presence of the identified organism and do not rule out co-infection with other viruses.

Negative results in the setting of a respiratory illness may be due to infection with pathogens not detected by this test, or lower respiratory tract infection that may not be detected by an NP specimen. Negative results do not preclude infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results for other organisms identified by the test may require additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence and radiography) when evaluating a patient with possible respiratory tract infection.

The Ezplex® Viral Respiratory Real-time PCR Test is intended for use by trained operators who are proficient in performing diagnostic tests.

II. Summary and Explanation

Acute respiratory infections are common conditions that generally show minor symptoms, but depending on the patient's condition, can also cause severe respiratory problems that require hospitalization.[1] In particular, respiratory infections can exhibit severe clinical conditions in children with relatively weak immune systems, including viruses such as Adenovirus, and Coronavirus. They are generally similar in symptoms, and overlapping infections are common, making it difficult to diagnose causative pathogens in many cases and clinically difficult to

identify. Conventional serological tests are less sensitive and take more time to obtain results.[2] Molecular diagnostics using the Nucleic Acid Amplification Tests (NAAT) can compensate for the shortcomings of traditional screening methods by enabling early detection of viruses that cause respiratory infections.[3] In particular, the Multiplex Real-time PCR method is useful for diagnosing respiratory infectious diseases that require rapid identification of various viral causative agents by allowing simultaneous examination of different types of infections.[4] As a result, a product that can accurately detect virus respiratory infections has been developed using the Real-time PCR.

III. Principles of the Procedure

The Ezplex® Viral Respiratory Real-time PCR Kit is a real-time polymerase chain reaction test. Each respiratory pathogen's primer and probe set(s) is designed to detect nucleic acids (RNA) from Influenza A, Influenza H1N1, Influenza H3N2, Pandemic H1N1/09 virus, Influenza B, Respiratory syncytial virus A, Respiratory syncytial virus B, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Adenovirus, Metapneumovirus, Enterovirus, Bocavirus, Rhinovirus A/B/C, Coronavirus 229E, Coronavirus OC43, and Coronavirus NL63. The Ezplex® Viral Respiratory Real-time PCR Kit is a test developed using Real-time PCR to quickly detect types of respiratory viruses. During the PCR process, if the RNA of these microorganisms is amplified during each cycle, a hydrolysis probe method is used to determine whether genes are amplified by fluorescence of these amplified products. In addition, during the last PCR cycle, two pathogens are isolated from the same fluorescent channel using a Tm value in which the target changes from double strand to single strand during the melting stage.

The Ezplex® Viral Respiratory Real-time PCR Kit consists of 3 panels of primer-probe mixtures as shown in the table below.

Panel	Target	Reporter Dye
I	Influenza A virus	FAM
	Influenza H1N1	HEX
	Influenza H3N2	HEX
	Respiratory syncytial virus A	TexasRed
	Respiratory syncytial virus B	TexasRed
	Pandemic H1N1/09 virus	Cy5
	Influenza B virus	Cy5
	Internal control	Quasar705
II	Parainfluenza virus 2	FAM
	Parainfluenza virus 3	HEX
	Parainfluenza virus 4	HEX
	Metapneumovirus	TexasRed
	Parainfluenza virus 1	Cy5
	Enterovirus	Cy5
	Internal control	Quasar705
III	Coronavirus 229E	FAM
	Bocavirus	HEX
	Coronavirus OC43	HEX
	Coronavirus NL63	TexasRed
	Adenovirus	TexasRed
	Rhinovirus A/B/C	Cy5
	Internal control	Quasar705

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 nucleic acid 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 can be added to each clinical sample during extraction preparation or into the reaction master mixture during PCR preparation procedure.

IV. Kit Components and Packaging Specifications

Catalog Number: GNT-2007-1 100 Tests/Kit

No.	Component Name*	Volume 100 Tests/Kit	Main Ingredients
1	RQ Mixture I	1 vial, 1000uL	DNA Taq polymerase, Reverse Transcriptase, dNTPs with dUTP, Magnesium Chloride, Potassium, Uracil N-glycosylase
2	RQ Mixture II	1 vial, 1000uL	DNA Taq polymerase, Reverse Transcriptase, dNTPs with dUTP, Magnesium Chloride, Potassium, Uracil N-glycosylase
3	RQ Mixture III	1 vial, 1000uL	DNA Taq polymerase, Reverse Transcriptase, dNTPs with dUTP, Magnesium Chloride, Potassium, Uracil N-glycosylase
4	Panel I Probe Primer	1 vial, 500 uL	Tris-HCl, EDTA, 10 - 12% oligonucleotide primers specific for respiratory pathogens, 3.8 - 5 % fluorescent-labeled oligonucleotide probe specific for respiratory pathogens
5	Panel II Probe Primer	1 vial, 500 uL	Tris-HCl, EDTA, 10 - 12% oligonucleotide primers specific for respiratory pathogens, 3.8 - 5 % fluorescent-labeled oligonucleotide probe specific for respiratory pathogens
6	Panel III Probe Primer	1 vial, 500 uL	Tris-HCl, EDTA, 10 - 12% oligonucleotide primers specific for respiratory pathogens, 3.8 - 5 % fluorescent-labeled oligonucleotide probe specific for respiratory pathogens
7	Positive control I	1 vial, 30 uL	Tris-HCl, EDTA, 60 - 70% synthesized DNA control specific for respiratory pathogens
8	Positive control II	1 vial, 30 uL	Tris-HCl, EDTA, 60 - 70% synthesized DNA control specific for respiratory pathogens
9	Positive control III	1 vial, 30 uL	Tris-HCl, EDTA, 60 - 70% synthesized DNA control specific for respiratory pathogens
10	Negative control	1 vial, 1000 uL	Double Distilled Water
11	Internal control	1 vial, 1000 uL	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. GNT-2007-3)

V. Materials Required But Not Provided

No.	Name	CAT No.	Manufacturer
1	Any applicable 96 well PCR plate plastics for Bio-Rad CFX96 DX system	MLL9651	Bio-Rad
2	Any applicable PCR plate sealing film for Bio-Rad CFX96 Dx system	MSB1001	Bio-Rad
3	Any applicable barrier tips appropriate for molecular testing	-	-
4	Any applicable pipettes with several volumes appropriate for molecular testing	-	-
5	Any applicable 1.5mL or higher distilled vials	-	-
6	QIAamp® DSP Virus Spin Kit	61704	Qiagen
7	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® Viral Respiratory Real-time PCR Kit:

- CFX96 Dx Real-time PCR Instrument with 1.6 or 3.1 or later versions of CFX Manager (Bio-Rad)

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.
- The product is valid only if it is not more than 6 months from date of opening.
- Completely thaw the reagents before use.
- Repeated thawing and freezing should be avoided.

VIII. Warning and Precautions

- For *in vitro* diagnostic use. This test should only be used by clinical experts such as clinical pathologists and medical technologists.
- This product is intended only for the detection of nucleic acid from Influenza A, Influenza H1N1, Influenza H3N2, Pandemic H1N1/09 virus, Influenza B, Respiratory syncytial virus A,

Respiratory syncytial virus B, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus 4, Adenovirus, Metapneumovirus, Enterovirus, Bocavirus, Rhinovirus A/B/C, Coronavirus 229E, Coronavirus OC43, and Coronavirus NL63, not for any other bacteria or pathogens.

- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines [5].
- 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.
- Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of being infected with any respiratory pathogens.
- 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 bacteria, 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 Virus Spin Kit can be used with the Ezplex® Viral Respiratory Real-time PCR Kit for nucleic acid extraction.
- Only the Bio-Rad CFX Dx Real-time PCR Instrument can be used with the Ezplex® Viral Respiratory Real-time PCR Kit. This instrument should be calibrated regularly according to instrument's instructions to eliminate cross-talks between channels.
- The Ezplex® Viral Respiratory Real-time PCR 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.

IX. Collection, Storage and Shipment of Specimens

Only nasopharyngeal swabs collected in VTM 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).

- Nasopharyngeal swab (NP): Insert a swab into nostril parallel to the palate. Swab should reach depth equal to distance for nostrils to outer opening of the ear. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it.

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 respiratory viral infection should be conducted in consultation with a healthcare provider. 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 -70ºC or colder.

C. Shipping

Specimens should 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 and 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) and other respiratory virus[1].

D. Specimen Preparation

- It is recommended that nucleic acid shall be isolated and purified from nasopharyngeal swabs using the Qiagen QIAamp® DSP Virus Spin Kit.
- Ensure homogeneous mixing of prepared specimens.
- Utilize 140 µL of clinical sample and elute with 50 µL of Buffer AVE from the QIAamp® DSP Virus Spin 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 Virus Spin Kit Handbook for the protocol for extracting RNA using the QIAamp® DSP Viral RNA Mini Kit.

X. Test Procedure

A. Nucleic Acid Extraction

It is recommended that QIAamp DSP Virus Spin Kit (Qiagen) shall be used for RNA extraction and users shall follow the protocol included in the Kit Instructions for Use. Prior to the start of the nucleic acid extraction, 10 µL of Internal control (IC) can be directly added to the clinical sample (200 µL of sample) to verify successful extraction and PCR. 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

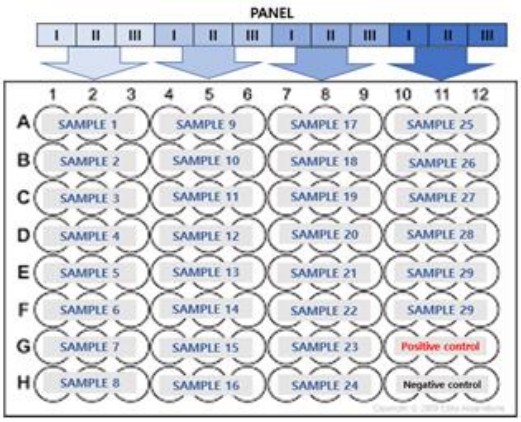
1. Reagent Master Mix Solution Preparation

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

Table 1. PCR Master Mixture Solutions (unit: uL)

Component	Volume (uL) per Specimen/Control
RQ Mixture (Panels I ~ IV)	10
Probe Primer (Panels I ~ IV)	5
Total	15

b. Pipette 15µL of each of the PCR master mix solutions into 96 well PCR plate or 8-cap strip. Add 5µL of the sample (nucleic acid) into 96 well PCR plate or 8-cap strip. See illustration below.



c. Each batch of samples tested should include the following controls: positive controls and negative control.

2. PCR Instrument Set Up

a. Set up the Bio-Rad PCR Instrument according to its Instrument Reference Guide/Manual using the cycling specifications below:

Step	Temperature / Time	Cycle
Hold	25 ℃ / 2 min	1 Cycle
	53 ℃ / 30 min	
	95 ℃ / 10 min	
Cycle	95 ℃ / 20 sec	40 Cycles
	55 ℃ / 1 min	
Hold	95 ℃ / 3 min	1 Cycle
	35 ℃ / 5 min	
Melting	+0.5 ℃ / 5 sec	35 ℃ ~ 85 ℃

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

Threshold	FAM	Cy5	HEX	Quasar705	Texas Red
Amplification curve	500	300	500	100	500
Melting curve values	N/A	43 - 45	48 - 50	N/A	38 - 40

c. Add the prepared 96-well plate or tubes to the Bio-Rad PCR Instrument and run according to its Instrument Guide/Manual.

XI. Quality Control

Positive Controls and Negative Control are provided with the Kit and should be run with each batch of specimens. Internal controls [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. These controls can be added to clinical specimens before RNA extraction procedure to monitor RNA extraction/recovery or transcription or this control can be added to the reaction master mix during PCR preparation procedure to monitor DNA transcription.

XII. 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 controls.

Panel	Target	Dye	Interpretation of results
I	Influenza A virus	FAM	Amplification detected
	Influenza H1N1	HEX	Amplification detected and melt Cts between 56 - 60
	Influenza H3N2	HEX	Amplification detected and melt Cts between 64 - 73
	Respiratory syncytial virus A	TexasRed	Melt Cts between 39 - 54
	Respiratory syncytial virus B	TexasRed	Amplification detected and melt Cts between 62 - 70
	Pandemic H1N1/09 virus	Cy5	Amplification detected and melt Cts between 55 - 60
	Influenza B virus	Cy5	Amplification detected and melt Cts between 66 - 74
	Internal control	Quasar705	Amplification detected
II	Parainfluenza virus 2	FAM	Amplification detected
	Parainfluenza virus 3	HEX	Amplification detected and melt Cts between 55 - 67
	Parainfluenza virus 4	HEX	Amplification detected and melt Cts between 68 - 72
	Metapneumovirus	TexasRed	Amplification detected
	Parainfluenza virus 1	Cy5	Amplification detected and melt Cts between 53 - 66

Panel	Target	Dye	Interpretation of results
	Enterovirus	Cy5	Amplification detected and melt Cts between 68 - 74
	Internal control	Quasar705	Amplification detected
III	Coronavirus 229E	FAM	Amplification detected
	Bocavirus	HEX	Amplification detected and melt Cts between 67 - 74
	Coronavirus OC43	HEX	Amplification detected and melts Cts between 56 - 66
	Coronavirus NL63	TexasRed	Amplification detected and melt Cts between 56 - 63
	Adenovirus	TexasRed	Amplification detected and melt Cts between 63.2 - 77
	Rhinovirus A/B/C	Cy5	Amplification detected
	Internal control	Quasar705	Amplification detected

XIII. Genetree Viewer Software Analysis

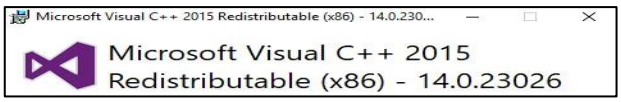
NOTE: Please contact 'technicalsupport@smlgenetree.com' or 'genetree@genetree.co.kr' to acquire 'Genetree Viewer' software prior to running the Ezplex® Viral Respiratory Real-time PCR Test.

A. Software Installation

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

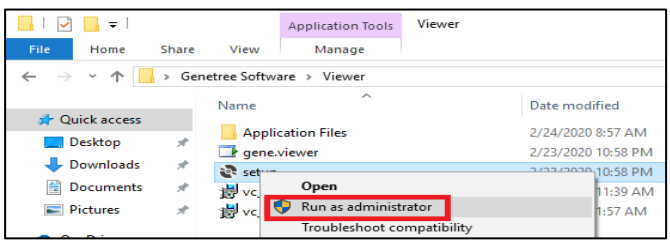
NOTE: This package is contained in the Genetree Viewer installation zip file.

NOTE: The Microsoft Visual C++ 2015 Redistributable(x86) is saved as "vc_redist.x86".

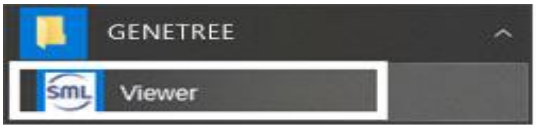


2. After pre-installation step, click on **'Run as Administrator'** in the file **'Setup.exe'** in the installation folder of **'Genetree Viewer'** and follow the instructions in the setup wizard.

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



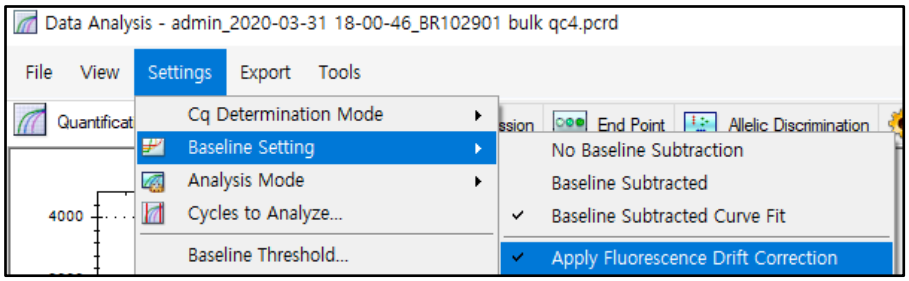
3. If the installation is completed, the run file of analysis software can be found in the 'Start menu' as below.



B. Software Analysis (Bio-Rad CFX96 Dx Real-time PCR Instrument)

1. Check that PCR is finished and that the threshold is applied correctly. Then click to enable apply fluorescence drift correction in the CFX96 Dx Manager software.

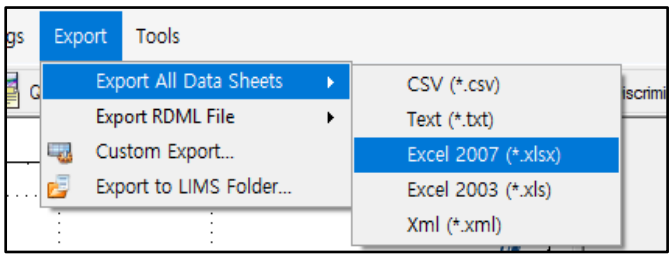
NOTE: This configuring step is only needed to CFX Manager software 3.1 version. In case of use CFX manager 1.6, the step '①' and '②' can be skipped.



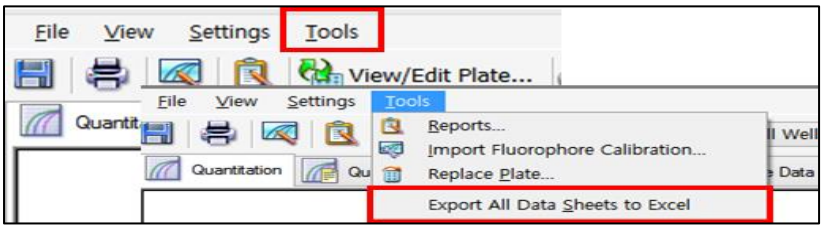
2. Click **'Export All Data Sheets'** from CFX Manager software's **'Tool'** menu to convert the test data into an excel spreadsheet (Create a folder and save the file in it).

NOTE: Select Excel 2003 or Excel 2007 according to the Excel specifications.

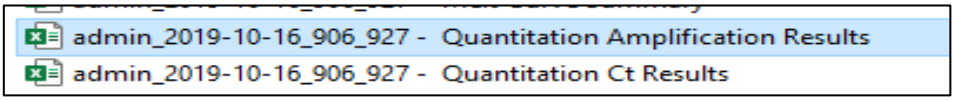
<CFX Manager software: 3.1 version>



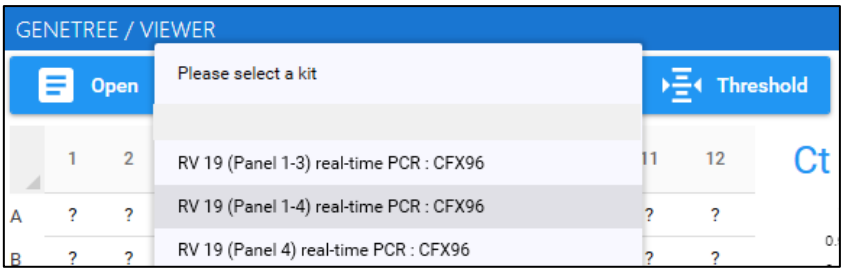
<CFX Manager software: 1.6 version>



3. Specify a folder to save the file you want, make New Folder and save the file in it.
4. 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.'**

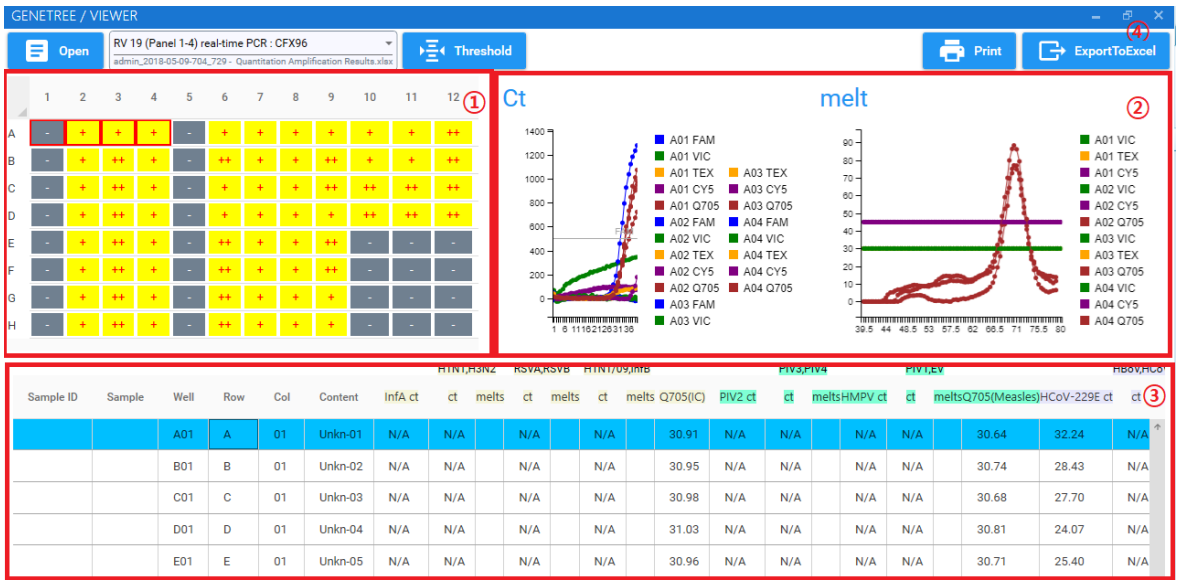


5. Click **'Please select a kit'** menu at the top of the screen and select an appropriate item for the tested panel



C. General Description of the Software and Example Screens

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.



XIV. Limitations

- The Ezplex® Viral Respiratory Real-time Kit has been validated for use with Nasopharyngeal swab sample run on the Bio-Rad CFX96 DX Real-Time PCR Instrument and utilizing the Qiagen QIAamp DSP Virus Spin Kit.
- 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 the presence of other respiratory pathogens not detected with this kit 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.
- Interpretation of results must account for the possibility of false negative and false positive results.
- A negative result for any PCR test does not conclusively rule out the possibility of infection.

XV. Performance Evaluation

A. Analytical Sensitivity (Limit of Detection)

Standard materials of each of the 19 types of respiratory pathogens were serially diluted into 5 concentrations (100, 10, 1, 0.5, and 0.1 copies/μL). The test was performed 24 times on each concentration for each pathogen. The limit of detection is calculated as below using probit analysis of 95% positive rate.

Panel	Target	Limit of Detection (Copies/uL)	Panel	Target	Limit of Detection (Copies/uL)
I	Influenza A virus	0.756	III	Coronavirus 229E	9.199
	Influenza H1N1	9.205		Bocavirus	8.753
	Influenza H3N2	9.476		Coronavirus OC43	9.205
	Respiratory syncytial virus A	4.741		Coronavirus NL63	47.253
	Respiratory syncytial virus B	0.925		Adenovirus	47.385
	Pandemic H1N1/09 virus	9.205		Rhinovirus A/B/C	46.428
	Influenza B virus	9.285			
II	Parainfluenza virus 1	9.358			
	Parainfluenza virus 2	89.276			
	Parainfluenza virus 3	9.477			
	Parainfluenza virus 4	94.73			
	Metapneumovirus	8.821			
	Enterovirus	0.925			

B. Analytical Specificity

1. Cross Reactivity

31 species of microorganisms with potential cross reactivity were tested in triplicate. No cross reactivity was observed.

Microorganisms
Candida glabrata, Candida tropicallis, Clostridium difficile, Corynebacterium amycolatum, Corynebacterium diphtheriae, Corynebacterium glutamicum, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Klebsiella oxytoca Klebsiella pnuemoniae subsp pneumoniae, Lactobacillus crispatus, Lactobacillus gallinarum, Lactobacillus vaginalis Nocardia sp., Peptostreptococcus anaerobius, Prevotella bivia, Prevotella disiens, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus anginosus subsp anginosus, Streptococcus constellatus subsp constellatus, Streptococcus gordonii, Streptococcus mutans, Streptococcus oralis, Streptococcus parasanguinis, Streptococcus salivarius, Streptococcus sanguinis, Veillonella parvula

2. Interference

The effect of interfering substances Albumin (0.24g/mL), Hemoglobin (0.2g/mL), Bilirubin (0.05mg/mL), Xylometazoline (1 mg/mL), and Mupirocin (0.67mg/mL) on assay performance was evaluated. Samples containing these substances were tested in triplicate with and without respiratory virus materials diluted in low concentration. No interference was observed as seen by coefficient of variation (CV) values which were less than 5% in all cases.

Controls	Interfering Substances									
	Albumin		Hemoglobin		Bilirubin		Xylometazoline		Mupirocin	
	Included	Not Included	Included	Not Included	Included	Not Included	Included	Not Included	Included	Not Included
Respiratory pathogens	Positive (CV < 5%)	Positive (CV < 5%)	Positive (CV <5%)	Positive (CV <5%)	Positive (CV <5%)	Positive (CV <5%)	Positive (CV <5%)	Positive (CV <5%)	Positive (CV <5%)	Positive (CV < 5%)
Negative control	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

C. Analytical Precision

1. Reproducibility

Reference materials for each of the respiratory pathogens were diluted into a middle concentration (100 X LOD) and a low concentration (10 X LOD). These were tested together with negative samples (D.W) using one Lot by 2 investigators from 2 test sites and each investigator testing 20 times repeatedly. The detection results were same across the tests with CV < 5%, and it was confirmed that results did not differ between investigators.

2. Repeatability

Reference materials for each of the respiratory pathogens were diluted into a middle concentration (100 X LOD) and a low concentration (10 X LOD). They were tested together with negative samples (D.W) using 3 Lots by 1 investigator and the investigator testing 20 times repeatedly, 4 times a day for 5 days. All the detection results were the same with CV < 5%, and it was confirmed that results did not differ within a test, between tests and between lots.

D. Clinical Evaluation

Clinical performance was evaluated by a clinical laboratory institution in the Republic of Korea using left-over nasopharyngeal swabs that were previously tested with products by Ministry of Food and Drug Safety (Republic of Korea). The results from testing of individual specimens are shown as follows:

Target	N	Positive agreement (95% CI)	N	Negative agreement (95% CI)
Influenza A virus	144	100 % (97.5 ~ 100%)	50	100 % (92.9 ~ 100%)
Influenza H1N1	43	100 % (91.8 ~ 100%)	50	100 % (92.9 ~ 100%)
Influenza H3N2	48	95.8 % (85.7 ~ 99.5%)	50	100 % (92.9 ~ 100%)
Pandemic H1N1/09 virus	43	100 % (91.8 ~ 100%)	50	100 % (92.9 ~ 100%)
Influenza B virus	43	100 % (91.8 ~ 100%)	50	100 % (92.9 ~ 100%)
Respiratory syncytial virus A	73	100 % (95.1 ~ 100%)	50	100 % (92.9 ~ 100%)
Respiratory syncytial virus B	44	100 % (92.0 ~ 100%)	50	100 % (92.9 ~ 100%)
Parainfluenza virus 1	58	100 % (93.8 ~ 100%)	50	100 % (92.9 ~ 100%)
Parainfluenza virus 2	43	100 % (91.8 ~ 100%)	50	100 % (92.9 ~ 100%)
Parainfluenza virus 3	85	100 % (95.8 ~ 100%)	50	100 % (92.9 ~ 100%)
Parainfluenza virus 4	40	100 % (91.2 ~ 100%)	50	100 % (92.9 ~ 100%)
Adenovirus	66	98.5 % (91.8 ~ 100%)	50	100 % (92.9 ~ 100%)
Metapneumovirus	152	100 % (97.6 ~ 100%)	50	100 % (92.9 ~ 100%)
Enterovirus	42	100 % (91.6 ~ 100%)	50	100 % (92.9 ~ 100%)
Bocavirus	125	97.6 % (93.1 ~ 99.5%)	50	100 % (92.9 ~ 100%)
Rhinovirus A/B/C	107	100 % (96.6 ~ 100%)	50	100 % (92.9 ~ 100%)
Coronavirus 229E	43	100 % (91.8 ~ 100%)	50	100 % (92.9 ~ 100%)
Coronavirus OC43	51	98.0 % (89.6 ~ 99.9%)	50	100 % (92.9 ~ 100%)
Coronavirus NL63	43	100 % (91.8 ~ 100%)	50	100 % (92.9 ~ 100%)

XVI. References

1. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, et al. Infectious diseases society of America/American thoracic society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007; 44(Suppl 2):S27-72.
2. Woo JH, Kang JM, Kim YS, Shin WS, Ryu JH, Choi JH, et al. A prospective multicenter study of community-acquired pneumonia in adults with emphasis on bacterial etiology. Korean J Infect Dis 2001;33:1-7.
3. Jung KS. Pneumonia in the elderly patients. Korean J Med 2008;75:129-40.
4. Murdoch DR. Molecular genetic methods in the diagnosis of lower respiratory tract infections. APMIS 2004;112:713-27.
5. WHO, Laboratory Biorisk Management Strategic Framework for Action 2012-2016, World Health Organization, 2012.

XVII. 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	-	

XVIII. 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**
- Email for Customer Support: **customersupport@smlgenetree.com**
- Tel: +82-2-2057-7900
- Fax: +82-70-7425-3950
- Mailing address: 225 Baumoe-ro, Seocho-gu, Seoul, 06740
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