Genematrix

INSTRUCTIONS FOR USE

NeoPlex™ RV-Panel A Detection Kit

REF

NR03A / NR03B

GENEMATRIX INC.

CE IVD

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NeoPlex™ RV-Panel A Detection Kit

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Multiplex Real-time PCR Reagents for Respiratory pathogens Detection
For professional *in vitro* diagnostic use only

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1. INTENDED USE

The 'NeoPlex™ RV-Panel A Detection Kit' Assay is a qualitative in vitro test for the simultaneous detection and confirmation of respiratory infection-causing pathogens (Influenza A including Influenza virus A H3N2(Flu A/H3N2) and Influenza virus A H1N1pdm09(Flu A/H1N1pdm09), Influenza virus B(Flu B), Respiratory Syncytial Virus A (RSV A) and Respiratory Syncytial Virus B (RSV B), Parainfluenza 1, 2 and 3 (PIV1, PIV2 and PIV3) and Adenovirus(AdV)) from nasopharyngeal swab specimens. This test kit is intended for professional use.

2. PRINCIPLE OF ASSAY

'NeoPlex™ RV-Panel A Detection Kit' is based on two major processes, isolation of nucleic acid from specimens and multiplex real-time amplification. Respiratory disease infection-causing pathogen's nucleic acid is extracted from a specimen, amplified in multiplex One-step real-time RT-PCR and detected using fluorescent reporter dye probes specific for the pathogens nucleic acid and Internal Control.

3. KIT CONTENTS

The 'NeoPlex™ RV-Panel A Detection Kit's components are shown in the table below.

1) NR03A (96 Tests)

Contents	Volume(96T)	Storage condition	Shelf life
5X RV-Panel A Buffer	400 µL x 1 Vial	Upper limit - 20 ℃	12 months (Before opening)
RV-Panel A Enzyme Mix	100 µL x 1 Vial		
4X RV-Panel A PPM	500 µL x 1 Vial		
RV-Panel A Positive Control(PC)	50 µL x 1 Vial		12 weeks (After opening)
RV-Panel A Internal Control(IC)	1 mL x 1 Vial		
DW(RNase-free Water)	1 mL x 1 Vial		

2) NR03B (50 Tests)

Contents	Volume(50T)	Storage condition	Shelf life
5X RV-Panel A Buffer	200 µL x 1 Vial	Upper limit - 20 ℃	12 months (Before opening)
RV-Panel A Enzyme Mix	50 µL x 1 Vial		
4X RV-Panel A PPM	250 µL x 1 Vial		
RV-Panel A Positive Control(PC)	25 µL x 1 Vial		12 weeks (After opening)
RV-Panel A Internal Control(IC)	0.5 mL x 1 Vial		
DW(RNase-free Water)	0.5 mL x 1 Vial		

4. COMPATIBLE INSTRUMENT

- CFX96™ Dx System (Bio-Rad, Cat No.1845097-IVD)

5. ADDITIONAL REQUIRED EQUIPMENT & MATERIALS

- CFX96™ Dx System (BioRad, Inc., Cat No. 1845097-IVD) or equivalent
- 0.2 ml 8-Tube PCR Strips without Caps, low profile, white (BioRad, Inc., Cat No. TLS0851)
- Optical Flat 8-Cap Strips for PCR Tubes (BioRad, Inc., Cat No. TCS0803)
- QIAamp DSP RNA Mini Kit (QIAGEN, Cat No.61904) or equivalent DNA extraction kit
- Pipettes set
- Micro Centrifuge
- Disposable powder-free gloves

6. KIT STORAGE AND STABILITY

- Store the kit below -20℃(-4°F).
- Kit materials are stable until the expiration date printed on the label under un-opened condition.
- Kit's shelf life is one (1) year.
- Please use the reagents within twelve (12) weeks after opening.

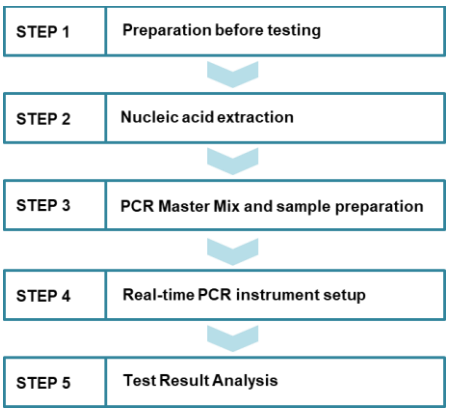
7. WARNINGS AND PRECAUTIONS

1. This device is intended for *in vitro* use only. Do not use the device for other purposes.
2. Wear personal protective equipment, such as gloves and lab coats when handling NeoPlex™ RV-Panel A Detection Kit and/or specimens.
3. Do not smoke, drink, or eat while handling NeoPlex™ RV-Panel A Detection Kit and/or samples.
4. Please be careful when handling samples to prevent infections of user and/or indirect contact to a person. Sample contains a risk of infections and unknown diseases.
5. Do not use reagents from different lots or from different tubes of the same lot.
6. If you do not frequently inspect the product, keep a kit in a refrigerator for a certain amount of time. Do not freeze/thaw over four times. Repeated frozen/thawed product may result in false negative and false positive results.
7. Be careful not to contaminate the product when extracting nucleic acid, amplifying PCR product, using Positive Control(PC). The use

of filter tips is recommended to prevent contamination of the product.

8. It is recommended that the sample or the Positive Control(PC) contained in the product to be frozen and stored separately from the freezer storing the product.
9. Use the sterilized consumable laboratory supplies. Do not reuse it.
10. Add the extracted nucleic acid sample and Positive Control(PC) into the reaction solution in a space separate form the PCR reaction solution preparation space.
11. Before using, read this instruction for use carefully.
12. Use calibrated measuring tools. (e.g. pipette)
13. Please check the expiration date before using the reagent.
14. Keep Positive Control separately when using to avoid contamination.
15. Before starting the PCR, make sure the lid is closed properly.
16. Dispose the product in accordance with local or national regulations.
17. Please consult with doctor about the test results.

8. TEST PROCEDURE



STEP 1. Preparation before testing

1) Preparation before testing

A. Prepare all the devices and reagent before use.
B. Place the kit under the room temperature at least 10 minutes before use.

⚠ Do not freeze/thaw over four (4) times.

2) Specimen Collection, Transportation and Storage

A. Specimens for use: Nasopharyngeal swab
B. It is recommended to process specimen within one (1) day after collection.
C. Store specimens at 2~8 °C (35.6~46.4°F) for no longer than seventy-two (72) hours. For pro-longed storage, Freeze at -20°C (-4°F) condition.
D. Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

⚠
- Use only the specimen type listed in the instruction manual.
- The specimen volume should be above 1ml.
- Wear eye protection, laboratory coats and disposable gloves when handling specimens.
- Specimens should be stored under the storage conditions above. Otherwise, the wrong test results can be obtained.
- Sample information should be recorded to avoid confusion.

STEP 2. Nucleic acid extraction

After pre-treatment, nucleic acid extraction can be done by auto mated purification system or using manual prep kits (QIAamp DS P RNA Mini Kit or equivalent).

1) Pre-treatment of the Specimen

Nasopharyngeal swab
Place the specimen at room temperature to normal temperature.
Vortex the sample for 20 seconds or more before use.

***** The Internal Control (RV-Panel A Internal Conotrl(IC)) is included in the kit. This allows the user to monitor the nucleic acid isolation procedure and the possibility of PCR inhibition.
Add 10μL of RV-Panel A Internal Control(IC) to the solution mixture or directly to the specimen if necessary.

2) For nucleic acid extraction, follow the manufacturer's protocol.

We recommend QIAamp DSP Viral RNA Mini Kit or equivalent nucleic acid extraction kit/automatic machine for nucleic acid extraction.

STEP 3. PCR Master Mix and sample preparation

1) Prepare the Master Mix

Contents	Volume per test
5X RV-Panel A Buffer	4 μL
RV-Panel A Enzyme Mix	1 μL
4X RV-Panel A PPM	5 μL
DW(RNase-free water)	5 μL
Total Volume	15 μL

Note: Calculate the required amount of each reagent based on the number of reactions (samples + controls).

2) Vortex and briefly centrifuge the PCR Master Mix.
3) Place 15 μL aliquots of the PCR Master mix into 0.2 ml PCR tubes and close the lids.
4) Add 5 μL of each nucleic acid sample to its respective tube.

Contents	1 test (Volume)
PCR Mixture Mix	15 μL
Nucleic acid sample	5 μL
Total Reaction Volume	20 μL

- It is recommended that the PCR mixture to be prepared just before use.
- Aerosol-resistant filter tips and tight gloves should be used when preparing samples.
- Take great care to avoid cross contamination.
- Defrost the reagents completely.
- Centrifuge the reagent tubes briefly to remove the drops from the inside of the lids.

5) Make the control amplification reactions.

- Negative Control(NC): Add 5 µL of DW(RNase-free Water) instead of nucleic acid samples to the tube.
- Positive Control(PC): Add 5 µL of RV-Panel A Positive Control(PC) instead of nucleic acid samples to the tube.

- Use a new pipette tip with each different sample.
- Avoid cross-contamination of PCR Master Mix and samples with Positive Control.
- For CFX96™ Dx System, do not label on the cap of the reaction tubes as fluorescence is detected through the cap.
- Centrifuge the PCR tube thoroughly for 30 seconds.

STEP 4. Real-time PCR instrument setup

1) Setting the PCR protocol.

PCR protocol should be set according to the table as below.

Segment	Tm(°C)	Time	Cycles
1	55	20 min	1
2	95	15 min	1
3	95	10 sec	40
4	65	90 sec	
5	73	10 min	1
6	55	30 sec	1
7*	Melting curve 55 ℃ ~ 90 ℃ (5s /0.5℃)*		

** Segment 7: Melting curve measurement*

For setting the PCR instrument, follow the Instruction for use from manufacturer of PCR instrument.

STEP 5. Test result analysis

Test results should be interpreted according to the '9. INTERPR ETATION OF TEST RESULTS' presented as below.

9. INTERPRETATION OF TEST RESULTS

For the analysis of the test result after PCR amplification, take the melting peak result (For CFX96™ Dx System check the 'Melt Peak' tab) and interpret the according to the following interpretation table.

1. Interpretation criteria for result analysis

Target	Dye	Melt Tm	Cut-off(RFU*)
Flu A	Cal Red 610	77.5 ± 1℃	≥ 100
Flu A/H3N2	FAM	76.5 ± 1℃	≥ 100
Flu A/ H1N1pdm09	FAM	86.5 ± 1℃	≥ 100
Flu B	HEX	85.5 ± 1℃	≥ 100
RSV A	HEX	66.5 ± 1℃	≥ 100
RSV B	HEX	75.5 ± 1℃	≥ 100
PIV1	Quasar 670	66.0 ± 1℃	≥ 100
PIV2	Quasar 705	81.0 ± 1℃	≥ 100
PIV3	Quasar 670	85.5 ± 1℃	≥ 100
AdV	Cal Red 610	84.0 ± 1℃	≥ 100
IC	Cal Red 610	66.0 ± 1℃	≥ 100

* RFU(–d(RFU)/dT): Relative fluorescence units

2. Interpretation of result

Target	IC		Result
+	+	Detected	Target is detected.
-	+	Not detected	Target is not detected.
-	-	Invalid	The negative (-) result of IC is the result of inhibition of PCR reaction due to the presence of a PCR inhibitor contained in the sample, and the sample is not suitable for the test. It is recommended to remove the PCR inhibitor and perform the nucleic acid extraction again. RV-Panel A Internal Control(IC) may be mixed with 10 µl during the extraction process.
+	-	Detected	If the nucleic acid concentration is high in the sample, IC signal may be attenuated. Dilute the template nucleic acid in distilled water and repeat the PCR with the diluted nucleic acid

*If only Influenza A (Flu A) virus is detected (and not the Flu A subtype such as H3N2 or H1N1pdm09), the subtype would be reported as unidentifiable and sequencing is recommended. If only the Flu A subtype is detected the sample indicates a very low viral load of influenza virus, the result would be reported as Flu A "Positive".

3. Application examples of clinical samples

No	FAM		HEX Cal Red 610			Quasar 670	
	H1N1pdm09	H3N2	Flu B	RSV A	RSV B	PIV1	PIV3
Sample 1	-	+	-	-	-	-	-
Sample 2	-	-	-	+	-	-	-
Sample 3	+	-	+	+	-	-	-
Sample 4	-	-	-	-	-	-	-
Sample 5	-	-	-	-	-	-	-
Positive Control	+	+	+	+	+	+	+
Negative Control	-	-	-	-	-	-	-

No	Quasar705	Cal Red 610		
	PIV2	Flu A	AdV	IC
Sample 1	-	+	-	+
Sample 2	-	-	-	+
Sample 3	-	-	-	+
Sample 4	-	-	-	+
Sample 5	-	-	-	-
Positive Control	+	+	+	+
Negative Control	-	-	-	-

No	Interpretation
Sample 1	Flu A, Flu A/H3N2 detected
Sample 2	RSV A detected
Sample 3	Flu A/H1N1pdm09, Flu B, RSV A detected
Sample 4	Not detected

Sample 5	Invalid
Positive Control	Positive(Valid)
Negative Control	Negative(Valid)

10. QUALITY CONTROL

NeoPlex™ RV-Panel A Detection Kit includes RV-Panel A Positive Control(PC) as positive control and DW(RNase-free water) as negative control. For all runs, valid test results must be obtained for both Positive and Negative control. Positive Control result must be Positive (Valid). Negative Control result must be Negative (Valid). If the positive and negative control results are consistently invalid, contact us for technical assistance.

11. TROUBLE SHOOTING

1. If the Internal control signal is not observed

Potential causes	Solution
Error in specimen collection	If both target and IC signal were not observed, recollect the specimen
Nucleic acid extraction failure	Carefully read the instruction for use of nucleic acid extraction kit and extract the nucleic acid from specimen again. IC may be mixed with 10 µl during the extraction process.
Incorrect PCR setting	Repeat the detection procedure with a correct setting
Incorrect PCR cycle or machine temperature	Check the PCR conditions and repeat the PCR under the correct setting if necessary
The fluorescence for data analysis does not comply with the protocol	Select the correct fluorescence for each target listed in this Instruction guide for data analysis
Leaving reagents at room temperature for a long time or incorrect storage condition	Check the storage conditions and the expiration date of the reagents and use a new kit
Presence of inhibitor	Dilute the template nucleic acid in distilled water (10-100x) and repeat the PCR with the diluted nucleic acid (If specimen is still present, restart from nucleic acid extraction procedure)
High load of pathogen's nucleic acid	Dilute the template nucleic acid in distilled water (10-100x) and repeat the PCR with the diluted nucleic acid

2. If signals are observed at the negative control/ false positive

Potential causes	Solution
Presence of cross contamination	Decontaminate all surfaces and instruments with sodium hypochlorite or ethanol. Use filter tips during the extraction procedure. Change tips among tubes. Repeat the nucleic acid extraction with the new set of reagents

3. If no signal is observed at the positive control/ false negative

Potential causes	Solution
Error in specimen collection	Recollect the specimen
Incorrect storage of the specimen	Recollect the specimen and repeat the whole process. Make sure the product is stored in recommended conditions
Error in nucleic acid extraction	Re-extract the nucleic acid
Incorrect PCR setting	Repeat the PCR with corrected setting
Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers for nucleic acid containing tubes and make sure to add nucleic acid into correct PCR tubes during detection process.
Incorrect PCR mixture	Check whether all components are added or not (If you use to pre-composed premix, should be reduce sensitivity) Each reagents should be used after homogenization and spin down reagent tube before put the real-time PCR

12. PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

1.1 Limit of Detection (LoD)

This study was conducted to determine the sensitivity by testing Liquid based cytology specimen. The proportion of positive results obtained from each concentration was subjected to 95% hit rate by probit analysis, and LoD of each target were obtained by performing 24 times of the tests.

Target		LoD
Flu A	Flu A/H3N2	2.84 X 10^{-2} PFU/mL
	Flu A/H1N1pdm09	6.14 X 10^{-1} PFU/mL
Flu A/H3N2		4.65 X 10^{-3} PFU/mL
Flu A/H1N1pdm09		5.33 X 10^{-2} PFU/mL
Flu B		2.67 X 10^{2} CEID50/mL
RSV A		7.46 X 10^{1} PFU/mL
RSV B		3.15 X 10^{-2} TCID50/mL
PIV1		1.18 X 10^{2} TCID50/mL
PIV2		2.53 X 10^{3} TCID50/mL
PIV3		1.34 X 10^{2} TCID50/0.2mL
AdV		3.84 X 10^{3} PFU/mL

1.2 Cut-off value

For the cut-off establishment, ΔRFU value was set to be 100 for all targets.

1.3 Inclusivity test

The inclusivity of NeoPlex™ RV-Panel A Detection Kit, were determined based on –in silico and wet test results. In the wet test, the subtypes for each targets were used in three (3) repeated tests, and the results showed 100% detection rate.

Subtype
Influenza A virus (H3N2)
Influenza A virus (H3N2), A/Hong Kong/8/68
Influenza A virus (H1N1), A/Virginia/ATCC1/2009
Influenza B virus, B/Maryland/1/59
Influenza B virus, B/Taiwan/2/62
Influenza B virus, B/Hong Kong/5/72
Influenza B virus, B/Allen/45
Influenza B virus, B/GL/1739/54
Human Respiratory syncytial Virus, A2001/3-12
Human respiratory syncytial virus A, Long
Human Respiratory syncytial virus 9320, complete genome
Human parainfluenza virus 1 HPIV-1, C35
Human parainfluenza virus 2 (HPIV-2), Greer
Human parainfluenza virus 3 NIH 47885
Human parainfluenza virus 3 HPIV-1, C243
Human adenovirus 1
Human adenovirus B Adenovirus type 3
Human adenovirus C Adenovirus type 2
Human adenovirus E Adenovirus type 4
Human adenovirus B Adenovirus type 11
Mastadenovirus, Human Adenovirus D type 8
Adenovirus Type 41 Culture Fluid
DNA from Human adenovirus 31 strain 1315

2. Analytical Specificity

2.1 Interference

Total eleven (11) substances, endogenous and exogenous source, were studied to determine their interfering effect and no interference reactions was found with the concentration as below.

No	Interfering substance	Concentration	Remark
1	Dexamethasone	1.53 µmol/L	Exogenous source
2	Zanamivir	3.3 mg/ml	
3	Oseltamivir	25 mg/ml	
4	Mupirocin	6.6 mg/ml	
5	Tobramycin	5 ug/ml	
6	Lidocaine	85.3 umol/L	
7	Guaifenesin	15.2 mmol/L	
8	L-Nicotine	6.1 umol/L	Endogenous source
9	mucin	50 ug/ml	
10	Blood	60 ug/ml	
11	Ethanol	7% v/v	

2.2 Cross reactivity

For analytical specificity, three (3) times of cross reactivity studies used fifty-eight(58) different pathogens similar with RI-pathogens and other pathogens. As a result, PCR amplification and cross reactivity were not observed with all the pathogens as below.

No	Manufacturer	Pathogen	Result
1	Zeptometrix	Streptococcus pyogenes	No Cross-reactivity
2	Zeptometrix	Streptococcus oralis	
3	Zeptometrix	Streptococcus mitis	
4	Zeptometrix	Streptococcus bovis	
5	Zeptometrix	Streptococcus anginosus	
6	ATCC	Fluoribacter bozemanae (Legionella bozemanae)	
7	ATCC	Legionella anisa	
8	Zeptometrix	Legionella longbeachae	
9	ATCC	Haemophilus parainfluenzae	
10	ATCC	Aggregatibacter aphrophilus	
11	ATCC	Haemophilus haemolyticus	
12	Zeptometrix	Bordetella bronchiseptica	
13	Zeptometrix	Bordetella holmesii	
14	Zeptometrix	Pseudomonas aeruginosa	
15	Zeptometrix	Klebsiella pneumoniae	
16	Zeptometrix	Acinetobacter baumannii	
17	ATCC	Haemophilus ducreyi	
18	ATCC	Lactobacillus acidophilus	
19	ATCC	Escherichia coli	
20	ATCC	Bacteroides fragilis	
21	ATCC	Enterobacter cloacae	
22	ATCC	Proteus mirabilis	
23	ATCC	Staphylococcus epidermidis	
24	ATCC	Enterobacter faecalis	
25	ATCC	Quantitative Synthetic Human bocavirus (HBoV) DNA	
26	ZEPTOMETRIX	Human metapneumovirus (hMPV) 9 Type A1	
27	ZEPTOMETRIX	Human metapneumovirus (hMPV) 3 Type B1	
28	ZEPTOMETRIX	Human metapneumovirus (hMPV) 27 Type B2	
29	ATCC	Human rhinovirus 1A	
30	ATCC	Human rhinovirus 1B	
31	ATCC	Human Rhinovirus 14	
32	KBPV	Human Rhinovirus A, Human Rhinovirus 21	
33	KBPV	Human Rhinovirus A, Human Rhinovirus 8	
34	KBPV	Human Rhinovirus A, Human Rhinovirus 42	
35	KBPV	Human Rhinovirus A, Human Rhinovirus 7	
36	ATCC	Human Coxsackievirus A 10	
37	ZEPTOMETRIX	Human Coxsackievirus A 16	
38	ATCC	Human Coxsackievirus A 21	
39	ATCC	Echovirus 4	
40	ATCC	Echovirus 20	
41	KBPV	Human Coxsackievirus B1	
42	KBPV	Human Coxsackievirus B2	
43	KBPV	Human Coxsackievirus B5	
44	KBPV	Echovirus 6	
45	KBPV	Echovirus 9	
46	KBPV	Parainfluenza virus 4a	
47	KBPV	Parainfluenza virus 4b	
48	ATCC	Human coronavrius 229E	
49	ZEPTOMETRIX	Human corona virus OC43	
50	ZEPTOMETRIX	coronavirus culture fluid (NL63)	
51	ATCC	Chlamydophila pneumoniae strain J-21	
52	ATCC	Mycpoplasma pneumoniae	
53	ATCC	Streptococcus pneumoniae	
54	ATCC	Legionella pneumophila subsp pneumophila	
55	ATCC	Heamophilus influenzae	
56	ATCC	Bordetella pertussis	
57	ATCC	Bordetella parapertussis	
58	ATCC	Moraxella (Branhamella) catarrhalis	

2.3 Carry-over & Cross-contamination

This study was performed to evaluate the carry-over and potential cross contamination effect. High concentrated positive sample and negative control sample were cross tested using same PCR instrument, and 100% negative results (72/72) (95% CI: 90.36%-100%) for each negative specimen were determined, respectively.

3. Precision

3.1 Repeatability

To evaluate the repeatability of NeoPlex™ RV-Panel A Detection Kit, Repeatability test was performed two runs per day, three replicates per run, during consecutive twenty days under same test conditions. Samples were tested using high, medium, low concentrations of positive samples and negative control. We confirmed that every test results are met the acceptance criteria: within 10% of CV, 100% agreement and the repeatability of NeoPlex™ RV-Panel A Detection Kit is acceptable.

3.2 Reproducibility

The reproducibility study was performed with four different conditions: for Between-lot (3 lots), Between-tester (3 testers), Between-instrument (3 instruments), and Between-site (3 sites). All results showed 100% agreements.

4. Clinical Evaluation

The clinical performance study was performed in the clinical laboratory with the specimen collected from various sources, such a hospitals or clinics. The comparable CE-marked product already available on EU market was used as reference test.

For clinical sensitivity and specificity, the test results were analyzed with 2x2 table, and summarized as below:

4.1 Clinical Accuracy (Clinical Sensitivity & Specificity)

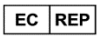
Target	Specimen type	Clinical sensitivity	Clinical specificity
Flu A	NPS	100% [95% CI 97.47-100]	100% [95% CI 99.23-100]
Flu A/H1N1pdm09	NPS	100% [95% CI 94.25-100]	100% [95% CI 99.34-100]

Flu A/H3N2	NPS	100% [95% CI 95.58-100]	100%([95% CI 99.32-100]
Flu B	NPS	98.67% [95% CI 92.83-99.76]	100% [95% CI 99.33-100]
RSV A	NPS	100% [95% CI 95.00-100]	100% [95% CI 99.33-100]
RSV B	NPS	100% [95% CI 96.60-100]	100% [95% CI 99.29-100]
PIV1	NPS	100% [95% CI 94.50-100]	100% [95% CI 99.34-100]
PIV2	NPS	100% [95% CI 93.24-100]	100% [95% CI 99.35-100]
PIV3	NPS	100% [95% CI 94.87-100]	100% [95% CI 99.33-100]
AdV	NPS	97.58% [95% CI 93.13-99.17]	100% [95% CI 99.27-100]

13. LIMITATION OF TEST

1) Results from this test must be correlated with the clinical history, epidemiological data, and other data of the patient available to the clinician.
2) If you do not use the samples and other specimens described in this manual, you may get inaccurate results.
3) Although the results of this test are negative, it is not advisable to exclude the possibility that the infection is actually present.
4) It is not excluded that this kit shows false positive results due to the presence of cross-contamination.
5) False negative results may occur due to polymerase inhibition. RV-Panel A Internal Control(IC) may help to identify any substance existing in the specimens interfering with nucleic acid isolation and PCR amplification.
6) This kit is for professional use only. Only trained healthcare provider can use this kit.

14. SYMBOLS

				
Catalogue number	Batch code	Date of manufacture	Use-by date	Distributor
				
In vitro diagnostic medical device	Upper limit of temperature	Caution	Consult instruction for use	Importer
				
Manufacturer	Contains sufficient for <n> tests	Authorized representative in the European Community	Conformity to European Directive 98/79/EC	Unique Device Identification

GeneMatrix Inc.
Manufacturing site
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CE IVD

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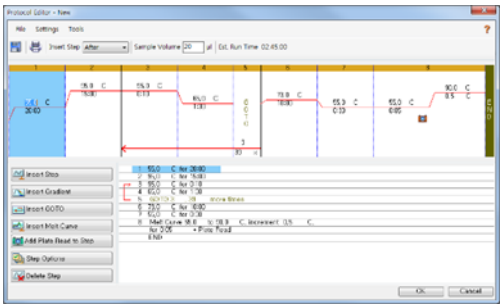
Appendix. PCR Instrument Operation

CFX96™ Dx System (Bio-Rad)

1. Protocol Setup

① Click File -> Protocol, Create a Protocol editor for PCR setup.

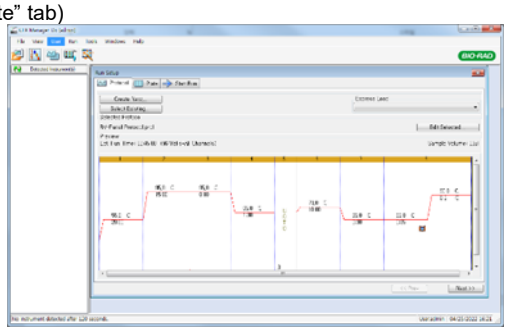
② The PCR condition is set as follows, and the sample volume set the 20 µL.



Segment	Tm(°C)	Time	Cycles
1	55	20 min	1
2	95	15 min	1
3	95	10 sec	40
4	65	90 sec	
5	73	10 min	1
6	55	30 sec	1
7*	Melting curve 55 ℃ ~ 90 ℃ (5s /0.5℃)		

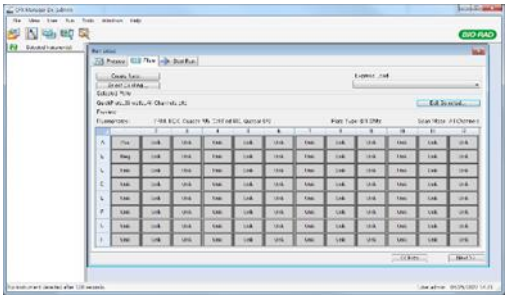
** Segment 7: Melting curve measurement*

③ After setting the PCR protocol, an Experiment Setup screen is created. Check the PCR protocol and click the “Next”. (Or click the “Plate” tab)

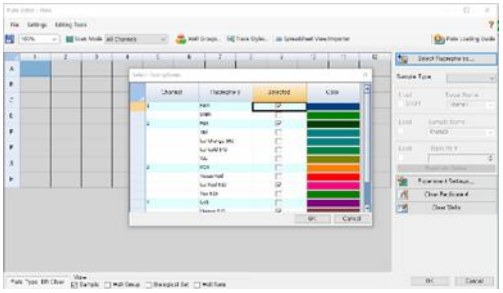


2. Plate Setup

① Click the “Create New” (or click the “Select Existing” load and existing plate for the experiment)



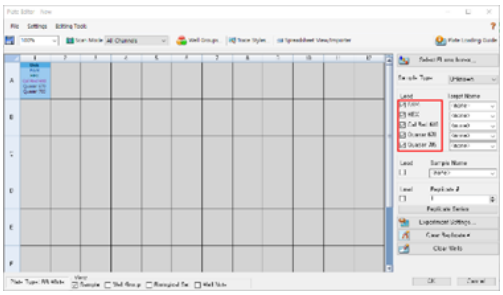
② Click the “Select Fluorophores”. Select the check box (FAM, HEX, Cal Red 610, Quasar 670, Quasar 705) for the fluorescent substance used for the experiment and click the OK button.



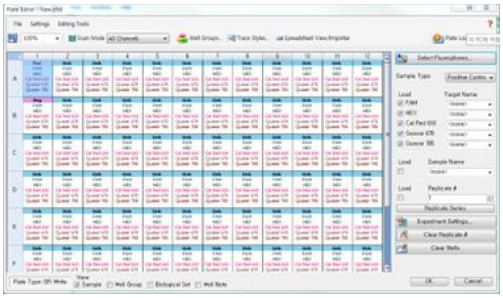
③ Select wells and select Sample Type from the drop-down menu.

Sample Type
‘Unknown’ : Clinical samples
‘Negative control’
‘Positive control’

④ Click the check box for the fluorescent substance (FAM, HEX, Cal Red 610, Quasar 670, Quasar 705) of the selected well.

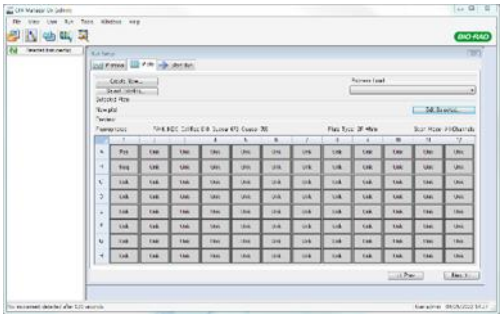


⑤ Click the “Settings” to set the plate type. (Settings -> Plate Type -> BR white)



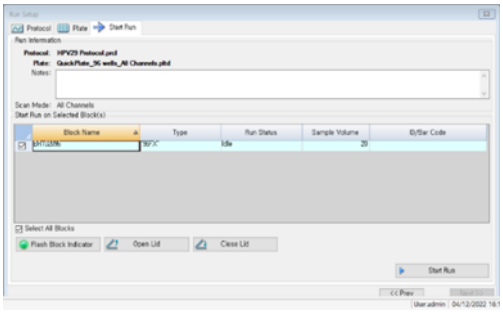
⑥ Click the “OK” and save a new Plate set-up file.

⑦ The Experimental Setup screen opens and checks the set plate. Click the “Next” (Or click the “Start Run” tab)



3. Start Run

① In the Experiment Setup Start Run Tab screen, click the “Close Lid” to close the lid of the equipment. (If the lid is closed, skip the step)



② Click the “Start Run”.

③ The operating file is stored in the user's designated folder, and the equipment begins to operate.

4. Pre-setting for Data analysis

① After the test, select the Melt curve to check the Melt Peak results.

② Select each analytical fluorescent substance (FAM, HEX, Cal Red 610, Quasar 670, Quasar 705) and set the threshold bar of Melt Peak to "0".