Genematrix

INSTRUCTIONS FOR USE

NeoPlex™ RB-8 Detection Kit

REF

NR02A / NR02B

GENEMATRIX INC.

CE 0123 IVD

NeoPlex™ RB-8 Detection Kit

Multiplex Real-time PCR Reagents for Respiratory Pathogens Detection

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Multiplex Real-time PCR Reagents for Respiratory Pathogens Detection

1. INTENDED USE

The 'NeoPlex™ RB-8 Detection Kit' is a qualitative *in vitro* test for the simultaneous detection and confirmation of eight (8) respiratory infection causing pathogens including *Streptococcus pneumoniae(SP), Mycoplasma pneumoniae(MP), Chlamydophila pneumoniae(CP), Legionella pneumophila(LP*), Haemophilus influenzae(HI), Bordetella pertussis(BP), Bordetella parapertussis*(BPP), *Moraxella catarrhalis* (MC) from human nasopharyngeal swab and sputum specimens based on multiplex Real-time polymerase chain reaction(PCR) assay. This test is intended for professionals use.

* LP target is only applied to sputum specimen

2. PRINCIPLE OF ASSAY

'NeoPlex™ RB-8 Detection Kit' is based on two major processes, isolation of DNA from specimens and multiplex real-time amplification. RI-causing pathogens DNA is extracted from specimens, amplified in multiplex real-time PCR and detected using fluorescent reporter dye probes specific for RI-causing pathogens DNA and Internal Control. Internal Control(IC) serves as an amplification control for each individually processed specimen and to identify possible reaction inhibition.

3. KIT CONTENTS

The 'NeoPlex™ RB-8 Detection Kit' components are shown in the table below.

1) NR02A (96 Tests)

Contents	Volume(96T)	Storage condition	Shelf life
4X NeoPlex PCR Master Mix	500 µL x 1 Vial	Upper limit -20 ℃	12 months (Before opening) 7 months (After opening)
4X RB-8 PPM	500 µL x 1 Vial		
RB-8 Positive Control(PC)	50 µL x 1 Vial		
RB-8 Internal Control(IC)	1 mL x 1 Vial		
DW(DNase-free Water)	1 mL x 1 Vial		

2) NR02B (50 Tests)

Contents	Volume(50T)	Storage condition	Shelf life
4X NeoPlex PCR Master Mix	250 µL x 1 Vial	Upper limit -20 ℃	12 months (Before opening) 7 months (After opening)
4X RB-8 PPM	250 µL x 1 Vial		
RB-8 Positive Control(PC)	25 µL x 1 Vial		
RB-8 Internal Control(IC)	0.5 mL x 1 Vial		
DW(DNase-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 DNA Mini Kit (QIAGEN, Cat No.61304) or equivalent DNA extraction kit
- Pipettes set
- Micro Centrifuge
- Disposable powder-free gloves

6. KIT STORAGE AND STABILITY

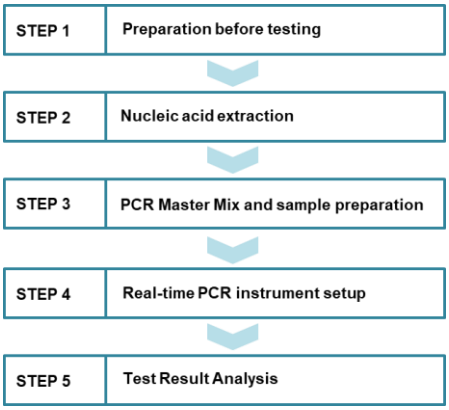
- Store the kit below -20℃(-4℉).
- Kit materials are stable until the expiration date printed on the label under un-opened condition.
- Kit's shelf life is one (1) year.
- Please use the reagents within seven (7) months after opening.

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™ RB-8 Detection Kit and/or specimens.
3. Do not smoke, drink, or eat while handling NeoPlex™ RB-8 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 five 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 from 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 on ice to dissolve the reagent at least 10 minutes before testing.

⚠ Do not freeze/thaw over five (5) times.

2) Specimen Collection, Transportation and Storage

A. Specimens for use: Nasopharyngeal swab and/or Sputum.

B. It is recommended to extract DNA from specimen immediately 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 below -20°C (-4°F).

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 0.5ml.
- 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, DNA extraction can be done by automated purification system or using manual prep kits (QIAamp DSP DNA Mini Kit or equivalent).

1) Pre-treatment of the Specimen

Nasopharyngeal swab	Sputum
Place the specimen at room temperature (19 ℃ ~ 25 ℃)	Place the specimen at room temperature (19 ℃ ~ 25 ℃)
Prepare the sample by vortexing for 20 seconds or more before use.	Add saline or PBS to the specimen (1 of specimen: 2 of saline or PBS) and vortex it for 1 minute.
	Leave it at room temperature for 20 minutes.
	Vortex it for 30 seconds.

2) Internal Control (Optional)

The Internal Control (RB-8 Internal Control(IC)) is included in the kit. This allows the user to monitor the nucleic acid isolation procedure and the possibility of PCR inhibition.

Urine: Add 10 µL of RB-8 Internal Control(IC) to each sample solution mixture.

3) DNA extraction

Follow the extraction kit's instruction for use to extract DNA.

We recommend QIAamp DSP DNA Mini Kit or equivalent DNA extraction kit for nucleic acid extraction.

STEP 3. PCR Master Mix and sample preparation

1) Prepare the PCR Master Mix

Contents	Volume per test
4X NeoPlex PCR Master Mix	5 µL
4X RB-8 PPM	5 µL
DW(DNase-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(DNase-free Water) instead of nucleic acid samples to the tube.
- Positive Control(PC): Add 5 µL of RB-8 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.
- When use CFX 96™ 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	50	4 min	1
2	95	15 min	1
3	95	20 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*

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 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*)
SP	FAM	66.5±1℃	≥ 80
LP	FAM	77.5±1℃	≥ 100
BP	FAM	85±1℃	≥ 100
HI	HEX	70±2℃	≥ 100
MC	HEX	79.5±1℃	≥ 100
MP	Cal Red 610	66.5±1℃	≥ 100
CP	Cal Red 610	75±1℃	≥ 100
BPP	Quasar 670	77±1℃	≥ 100
IC	Quasar 670	63.5±1℃	≥ 100

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

2. Interpretation of result

Target	IC		Result
+	+	Detected	Detection of pathogens causing respiratory infections
-	+	Not detected	Target not detected
-	-	Invalid	- (-) results of IC may be caused by the presence of a PCR inhibitor contained in the specimen or sample that are not suitable for the test. - It is recommended to remove the PCR inhibitor and perform it again from the extraction process. - If IC is not detected again after the retest, the RB-8 IC contained in the product can be added to the specimen then nucleic acid extraction can be performed to confirm the presence of the PCR inhibitor.
+	-	Detected	- If the concentration of nucleic acid in the pathogen is high, IC signal may be inhibited. If the target pathogen is detected but no IC is detected, the target pathogen is determined to be detected. If you want to check the IC, it is recommended to dilute the sample and retest it.

3. Application examples of clinical samples

No	FAM			HEX		Cal Red 610		Quasar 670		Interpretation
	SP	LP	BP	HI	MC	MP	CP	BPP	IC	
Sample 1	+	-	-	-	-	-	-	-	+	SP detected
Sample 2	-	-	-	+	-	+	-	-	+	HI, MP detected
Sample 3	-	+	-	-	+	+	-	-	+	LP, MC, MP detected
Sample 4	+	+	-	+	-		-	-	+	SP, LP, HI detected
Sample 5	+	-	-	-	-	-	-	-	+	SP detected
Sample 6	-	-	-	-	-	-	-	-	+	Not detected
Sample 7	-	-	-	-	-	-	-	-	-	Invalid
Positive Control	+	+	+	+	+	+	+	+	+	Positive (Valid)
Negative Control	-	-	-	-	-	-	-	-	-	Negative (Valid)

10. QUALITY CONTROL

NeoPlex™ RB-8 Detection Kit includes RB-8 Positive Control(PC) and DW(DNase-free Water) set. DW(DNase-free Water) is used as negative control. For all runs, valid test results must be obtain ed for both Positive and Negative control for NeoPlex™ RB-8 Det ection Kit. Positive Control result must be Positive (Valid). Negativ e Control result must be Negative (Valid). If the positive and nega tive 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 the both target and IC signal were not observed, recollect the specimen
Nucleic acid extraction failure	Read carefully the instruction for use of nucleic acid extraction kit and extract the nucleic acid from specimen again.
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 nasopharyngeal swab and sputum 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	Specimen type	LoD
SP	Sputum	$6.1x10^0$ copies/ul
	Nasopharyngeal swab	$6.1x10^0$ copies/ul
MP	Sputum	1.19 copies/ul
	Nasopharyngeal swab	1.17 copies/ul
CP	Sputum	$6.3x10^0$ copies/ul
	Nasopharyngeal swab	$6.1x10^0$ copies/ul
LP	Sputum	$1.18x10^0$ copies/ul
HI	Sputum	$1.13x10^0$ copies/ul
	Nasopharyngeal swab	$1.12x10^0$ copies/ul
BP	Sputum	$1.18x10^0$ copies/ul
	Nasopharyngeal swab	$1.16x10^0$ copies/ul
BPP	Sputum	$1.09x10^0$ copies/ul
	Nasopharyngeal swab	$1.1x10^0$ copies/ul
MC	Sputum	$1.19x10^0$ copies/ul
	Nasopharyngeal swab	$1.11x10^0$ copies/ul

1.2 Cut-off value

Target	Cut-off value(-d(RFU)/dT)
SP	≥ 80
MP	≥ 100
CP	≥ 100
LP	≥ 100
HI	≥ 100
BP	≥ 100
BPP	≥ 100
MC	≥100

2. Analytical Specificity

2.1 Interference

Total sixteen (16) substances, endogenous and exogenous source, were studied to determine their interfering effect and no interference reactions was found with the concentration as below.
The test concentration was selected referring the competitor devices on the market.

No	Types	Interfering substance	Concentration
1	Endogenous substances	Human Blood	2% v/v
2		mucin	50 µg/ml
3	Exogenous substances	Dexamethasone	1.53 µmol/L
4		Zanamivir	3.3 mg/ml
5		Oseltamivir	25 mg/ml
6		Mupirocin	6.6 mg/ml
7		Tobramycin	5 µg/ml
8		Lidocaine	85.3 µmol/L
9		Eucalyptol	10% v/v
10		Guaifenesin	15.2 mmol/L
11		L-Nicotine	6.2 µmol/L
12	Disinfecting/Cleaning Substances	Ethanol	7% v/v
13	Transport Medium	ESwab™ (Copan 482C)	N/A
14		UTM-RT (Copan 306C)	N/A
15		UTM(TS) (ASAN AM608-03)	N/A
16		Rest™ UTM (NobleBio UTM-001B)	N/A

2.2 Cross reactivity

For analytical specificity, three (3) times of cross reactivity studies using fifty-nine (59) different pathogens were performed. 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	Acinetobacter baumannii	
16	Zeptometrix	Neisseria meningitidis	
17	Zeptometrix	Klebsiella pneumoniae	
18	Vircell	Chlamydia trachomatis	
19	Vircell	Neisseria gonorrhea	
20	ATCC	Trichomonas vaginalis	
21	ATCC	Mycoplasma hominis	
22	ATCC	Mycoplasma genitalium	
23	ATCC	Ureaplasma urealyticum	
24	ATCC	Ureaplasma pavum	
25	ATCC	Gardnerella vaginalis	
26	ATCC	Haemophilus ducreyi	
27	ATCC	Candida albicans	
28	ATCC	Lactobacillus acidophilus	
29	ATCC	Escherichia coli	
30	ATCC	Bacteroides fragilis	
31	ATCC	Enterobacter cloacae	
32	ATCC	Proteus mirabilis	
33	ATCC	Staphylococcus epidermidis	
34	ATCC	Enterobacter faecalis	
35	BEI	Influenza A virus, A/New Caledonia/20/1999 (H1N1)	
36	ATCC	Influenza A virus (H1N1), A/FM/1/47	
37	ATCC	Influenza A virus (H1N1), A/PR/8/34	
38	ATCC	Influenza A virus (H1N1), A/New Jersey/8/76	
39	ATCC	Influenza A virus (H1N1), A/Denver/1/57	
40	BEI	Influenza A virus, A/Aichi/2/1968 (H3N2)	
41	ATCC	Influenza A virus (H3N2), A/Hong Kong/8/68	
42	ATCC	Influenza A virus (H3N2), A/Port Chalmers/1/73	
43	ATCC	Influenza A virus (H3N2), A/Victoria/3/75	
44	BEI	Influenza virus, A/California/04/2009 (H1N1)pdm09, Cell Isolate (Produeced in Cells)	
45	ATCC	Influenza A virus (H1N1), A/Virginia/ATCC1/2009	
46	ATCC	Influenza B virus, B/Maryland/1/59	
47	ATCC	Influenza B virus, B/Hong Kong/5/72	
48	BEI	Human Respiratory syncytial Virus, A2001/3-12, Purified from Hep-2 cells	
49	ATCC	Human respiratory syncytial virus A, Long	
50	ATCC	Human respiratory syncytial virus B, 9320	
51	ATCC	Human rhinovirus 1A	
52	ATCC	Human rhinovirus 1B	
53	ATCC	Human Rhinovirus 14	
54	ATCC	Human Coxsackievirus A 10	
55	Zeptometrix	Human Coxsackievirus A 16	
56	ATCC	Human Coxsackievirus A 21	
57	BEI	Enterovirus D68, US/MO/14-18947	
58	Zeptometrix	Human coronavrius 229E	
59	Zeptometrix	Human coronavrius OC43	

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 (128/128) (95% CI: 97.09%-100%) for each negative specimen were determined, respectively.

3. Precision

3.1 Repeatability

Repeatability was assessed by testing for twenty (20) different days, two (2) runs per day, three (3) cycles per run. Targets were set in three (3) levels of concentration, and 100% agreement was found determining the repeatability. The CV criteria, 10%, was met for all test results.

3.2 Reproducibility

To 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. The comparable CE-marked product already available on EU market was used as reference test.
For clinical sensitivity and specificity, test results were summarized as below:

4.1 Clinical Accuracy (Clinical Sensitivity & Specificity)

1) Nasopharyngeal

Nasopharyngeal	Clinical sensitivity	Clinical specificity
SP	98.36% [95% CI: 96.91-99.13]	99.89% [95% CI: 99.39-99.98]
MP	98.70% [95% CI: 96.25-99.56]	100% [95% CI: 99.69-100]
CP	100% [95% CI: 92.59-100]	100% [95% CI: 99.73-100]
HI	98.90% [95% CI: 97.21-99.57]	99.91% [95% CI: 99.49-99.98]
BP	100% [95% CI: 92.44-100]	100% [95% CI: 99.73-100]
BPP	100% [95% CI: 92.73-100]	100% [95% CI: 99.73-100]
MC	100% [95% CI: 97.82-100]	99.92% [95% CI: 99.56-99.99]

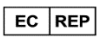
2) Sputum

Sputum	Clinical sensitivity	Clinical specificity
SP	98.45% [95% CI: 97.18-99.16]	99.72% [95% CI: 99.18-99.90]
MP	99.57% [95% CI: 97.59-99.92]	100% [95% CI: 99.74-100]
CP	100% [95% CI: 92.73-100]	100% [95% CI: 99.77-100]
HI	100% [95% CI: 92.73-100]	100% [95% CI: 99.77-100]
BP	99.42% [95% CI: 98.31-99.80]	99.83% [95% CI: 99.39-99.95]
BPP	100% [95% CI: 92.59-100]	100% [95% CI: 99.77-100]
MC	100% [95% CI: 92.86-100]	100% [95% CI: 99.77-100]

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. RB-8 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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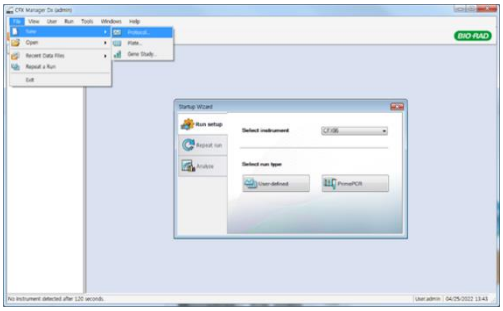
Appendix. PCR Instrument Operation

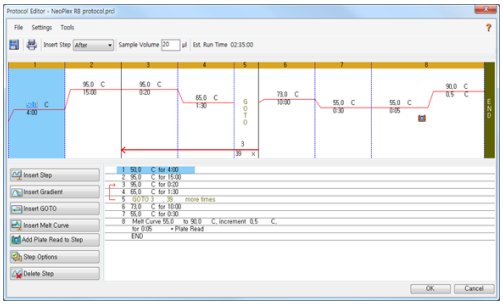
CFX96™ Dx System (Bio-Rad)

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.

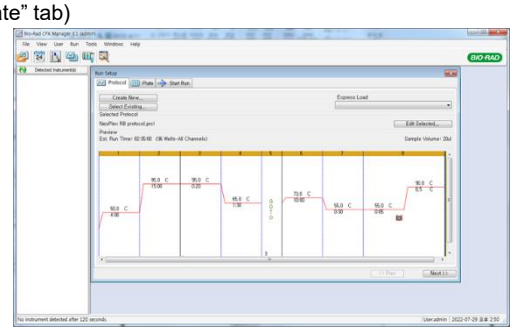




Segment	Tm(°C)	Time	Cycles
1	50	4 min	1
2	95	15 min	1
3	95	20 sec	40
4	65	90 sec	
5	73	10 min	1
6	55	30 sec	1
7*	Melting curve 55 ℃ ~ 90 ℃ (5s/0.5℃)*		

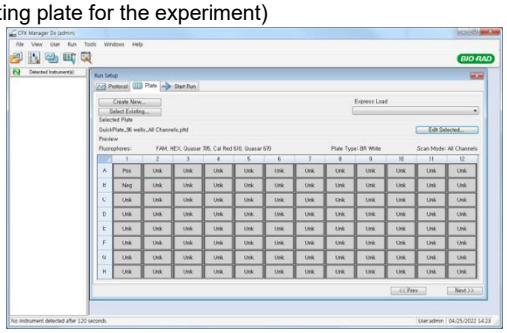
** Segment 7: Melting curve measurement*

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)

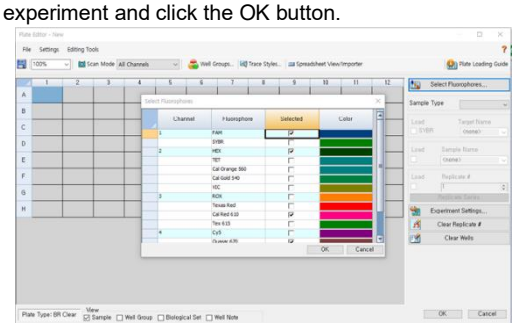


2. Plate Setup

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



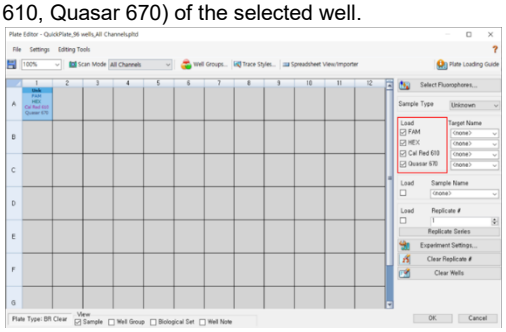
② Click the “Select Fluorophores”. Select the check box (FAM, HEX, Cal Red 610, Quasar 670) for the fluorescent substance used for the experiment and click the OK button.



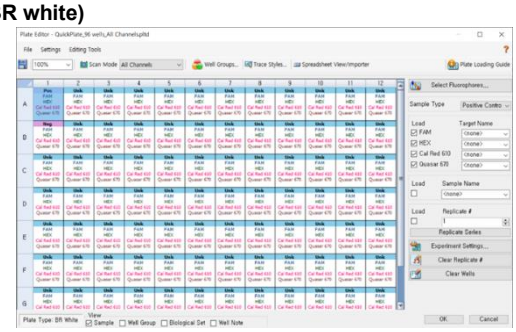
③ Select wells and select Sample Type from the drop-down menu.

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) of the selected well.

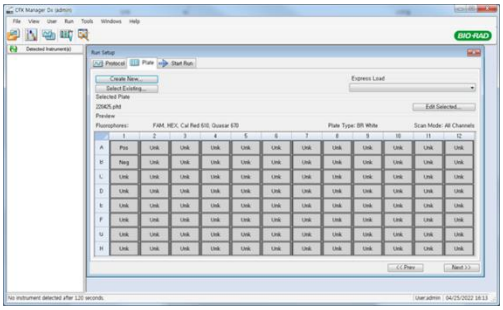


⑤ Click the “Settings” to set the plate type. (**Settings -> Plate Type -> BR white)**



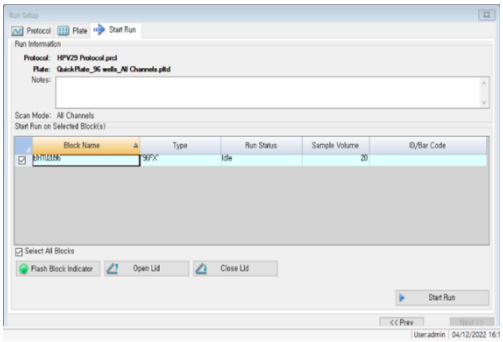
⑥ Click the “OK” and save a new Plate set-up file.

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) and set the threshold bar of Melt Peak to "0".