

# **INSTRUCTIONS FOR USE**

NeoPlex<sup>™</sup> RV-Panel B Detection Kit

REF

**NR04A / NR04B** 





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

# **Table of contents**

1. INTENDED USE	1
2. PRINCIPLE OF ASSAY	1
3. KIT CONTENTS	1
4. COMPATIBLE INSTRUMENT	1
5. ADDITIONAL REQUIRED EQUIPMENT & MATERIALS	1
6. KIT STORAGE AND STABILITY	1
7. WARNINGS AND PRECAUTIONS	1
8. TEST PROCEDURE	2
9. INTERPRETATION OF TEST RESULTS	3
10. QUALITY CONTROL	4
11. TROUBLE SHOOTING	4
12. PERFORMANCE CHARACTERISTICS	4
13. LIMITATION OF TEST	6
14. SYMBOLS	6
Annendix PCR Instrument Operation	7



REF NR04A / NR04B



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#### 1. INTENDED USE

The 'NeoPlex™ RV-Panel B Detection Kit' Assay is a qualitative *in vitro* test for the simultaneous detection and confirmation of res piratory infection-causing pathogens (Coronavirus 229E(229E), Coronavirus NL63(NL63), Coronavirus OC43(OC43), Coronavirus HKU 1(HKU1), Parainfluenza virus 4(PIV4), Human Enterovirus(HEV), Human Rhinovirus(HRV), Human Bocavirus(BoV) and Human Metap neumovirus(MPV)) from nasopharyngeal swab specimens. This test kit is intended for professional use.

#### 2. PRINCIPLE OF ASSAY

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

#### 1) NR04A (96 Tests)

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

#### 2) NR04B (50 Tests)

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

#### 4. COMPATIBLE INSTRUMENT

CFX96<sup>™</sup> Dx System (Bio-Rad, Cat No.1845097-IVD)

#### 5. ADDITIONAL REQUIRED EQUIPMENT & MATERIALS

- CFX96<sup>™</sup> 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 Viral RNA Mini Kit (QIAGEN,Cat No.61904) or equivalent nucleic acid extraction kit
- Pipettes set
- Micro Centrifuge
- · Disposable powder-free gloves

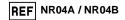
#### 6. KIT STORAGE AND STABILITY

- Store the kit below -20°C(-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

- This device is intended for *in vitro* use only. Do not use the device for other purposes.
- Wear personal protective equipments, such as gloves and lab coats when handling NeoPlex<sup>TM</sup> RV-Panel B Detection Kit and/ or specimens.
- Do not smoke, drink or eat while handling NeoPlex<sup>™</sup> RV-Pan el B Detection Kit and/or samples.
- 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.
- Do not use reagents from different lots or from different tubes of the same lot.
- If you do not frequently inspect the product, keep a kit in a r
  efrigerator for a certain amount of time. Do not freeze/thaw ov
  er four times. Repeated frozen/thawed product may result in f
  alse negative and false positive results.
- 7. Be careful not to contaminate the product when extracting nuc







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

leic acid, amplifying PCR product, using positive Control(PC, P ositive Control). The use of filter tips is recommended to prevent contamination of the product.

- It is recommended that the sample or the positive Control(PC, Positive Control) contained in the product to be frozen and st ored separately from the freezer storing the product.
- 9. Use the sterilized consumable laboratory supplies. Do not reus e it.
- 10. Add the extracted nucleic acid sample and positive Control(PC, Positive Control) 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 regul ations.
- 17. Please consult with doctor about the test results.

# 8. TEST PROCEDURE STEP 1 Preparation before testing STEP 2 Nucleic acid extraction STEP 3 PCR Master Mix and sample preparation STEP 4 Real-time PCR instrument setup STEP 5 Test Result Analysis

#### STEP 1. Preparation before testing

#### 1) Preparation before testing

- A. Prepare all the devices and reagent before use.
- B. Place the kit on ice when thawing components and preparing PCR Master Mix.
- C. After preparing PCR Master Mix, place them on ice.



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

#### 2) Specimen Collection, Transportation and Storage

- A. Specimens for use: Nasopharyngeal swab
- 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.
- 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, nucleic acid extraction can be done by auto mated purification system or using manual prep kits (QIAamp DS P Viral 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 B 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.

Add 10 µL of RV-Panel B 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

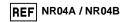
1) Prepare the Master Mix

Contents	Volume per test
5X RV-Panel B Buffer	4 μL
RV-Panel B Enzyme Mix	1 μL
4X RV-Panel B 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







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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  $\mu$ L of DW(RNase-free Water) instead of nucleic acid samples to the tube.
- Positive Control(PC): Add 5  $\mu$ L of RV-Panel B 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<sup>TM</sup> 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	40
5	73	10 min	1
6	55	30 sec	1
7*		urve 55 °C ~ 90 °C (0	

<sup>\*</sup> 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<sup>TM</sup> 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*)
229E	FAM	69.0 ± 2 °C	≥ 100
OC43	FAM	76.0 ± 2 °C	≥ 100
NL63	FAM	85.5 ± 2 °C	≥ 100
HEV	HEX	66.0 ± 2 °C	≥ 100
HRV	HEX	75.0 ± 2 °C	≥ 100
BoV	HEX	85.5 ± 2 °C	≥ 100
HKU1	Cal Red 610	76.5 ± 2 °C	≥ 100
PIV4	Cal Red 610	84.0 ± 2 °C	≥ 100
MPV	Quasar 670	76.0 ± 2 °C	≥ 100
IC	Cal Red 610	65.5 ± 2 °C	≥ 100

<sup>\*</sup> 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 B 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

#### 3. Application examples of clinical samples

No		FAM			FAM		
NO	229E	OC43	NL63	HEV	HRV	BoV	
Sample 1	+	-	-	-	+	-	
Sample 2	-	+	-	-	-	+	
Sample 3	-	-	-	-	+	-	
Sample 4	-	-	-	-	-	-	
Sample 5	-	-	-	-	-	-	
Positive control	+	+	+	+	+	+	
Negative control	-	-	-	-	-	-	

No	Cal Red 610			Quasar 670
NO	IC	HKU1	PIV4	MPV
Sample 1	+	-	+	=
Sample 2	+	-	-	=
Sample 3	-	-	-	=
Sample 4	+	-	-	-
Sample 5	-	-	-	-
Positive control	+	+	+	+
Negative control	-	-	-	-

No	Interpretation
Sample 1	229E, HRV, PIV4
Sample 2	OC43, BoV
Sample 3	HRV
Sample 4	Not detected
Sample 5	Invalid
Positive Control	Positive (Valid)
Negative Control	Negative (Valid)



REF NR04A / NR04B



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

#### 10. QUALITY CONTROL

NeoPlex™ RV-Panel B Detection Kit includes RV-Panel B Positive e Control(PC) as positive control and DW(RNase-free water) as n egative 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

NeoPlex<sup>™</sup> RV-Panel B Detection Kit includes RV-Panel B Positive Control(PC) and DW

#### 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	Read carefully 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 do 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 reagent should be used after homogenization and spin down reagent tube before putting 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 specimens.

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	
229E	8.01 X 10 <sup>-5</sup> TCID50/ml	
NL63	1.15 X 10 <sup>-3</sup> TCID50/ml	
OC43	1.04 X 10 <sup>-1</sup> TCID50/ml	
HKU1	2.09 X 10 <sup>1</sup> copies/µL	
PIV4	1.05 X 10 <sup>-1</sup> TCID50/ml	
HEV	6.11 X 10 <sup>-1</sup> TCID50/ml	
HRV	3.02 X 10 <sup>1</sup> TCID50/ml	
BoV	2.08 X 10 <sup>1</sup> copies/μL	
MPV	8.65 X 10 <sup>-1</sup> TCID50/ml	

#### 1.2 Cut-off value

For the cut-off establishment,  $\Delta \text{RFU}$  value was set to be 100 for all targets.

#### 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.

No	Interfering substance	Concentration	Remark
1	Human Blood	2% v/v	Endogenous
2	mucin	50 μg/ml	substances
3	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	Exogenous substances
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	Ethanol	7% v/v	Disinfecting/Cleani ng Substances
13	ESwab™ (Copan 482C)	N/A	
14	UTM-RT (Copan 306C)	N/A	Transport Madicus
15	UTM(TS) (ASAN AM608-03)	N/A	Transport Medium
16	Rest™ UTM (NobleBio UTM-001B)	N/A	

4 NR04-FN-R3



REF NR04A / NR04B



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

#### 2.2 Cross reactivity

For analytical specificity, three (3) times of cross reactivity studies used fourty-nine (49) 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.

BEI	No	Manufacturer	Pathogen	Result
BEI	1	BEI		
ATCC Influenza A virus (H3N2), A/Port Chalmers/1/73  4 ATCC Influenza B virus, B/Hong Kong/5/72  5 ATCC Human respiratory syncytial virus A, Long  6 ATCC Human Respiratory syncytial virus 9320, complete genome  7 ATCC Human adenovirus 1  8 ATCC Human parainfluenza virus 1 HPIV-1, C35  9 ATCC Human parainfluenza virus 2 (HPIV-2), Greer  10 ATCC Human parainfluenza virus 3 HPIV-1, C243  11 ATCC Streptococcus pneumoniae  12 ATCC Mycoplasma pneumoniae  13 VR-1435 Chlamydophila pneumophila subsp pneumophila  15 ATCC Heamophilus influenzae  16 ATCC Moraxella (Branhamella) catarrhalis  17 ATCC Bordetella pertussis  18 ATCC Bordetella parapertussis  19 ATCC Haemophilus haemolyticus  20 ATCC Aggregatibacter aphrophilus  21 ATCC Haemophilus haemolyticus  22 zeptometrix Pseudomonas aeruginosa  23 zeptometrix Acinetobacter baumannii  24 ATCC Legionella noisa  25 ATCC Legionella ongbeachae  27 zeptometrix Streptococcus progenes  28 zeptometrix Streptococcus progenes  29 zeptometrix Streptococcus progenes  20 zeptometrix Streptococcus progenes  31 zeptometrix Streptococcus sovis  31 zeptometrix Streptococcus sovis  31 zeptometrix Streptococcus sovis  32 zeptometrix Streptococcus sovis  33 zeptometrix Bordetella holmesii  34 HPKTCC Neisseria meningitides  35 zeptometrix Klebsiella pneumoniae  36 vircell Chlamydia trachomatis	2	BEI	Influenza virus, A/California/04/2009	1
5 ATCC Human respiratory syncytial virus A, Long 6 ATCC Human Respiratory syncytial virus 9320, complete genome 7 ATCC Human parainfluenza virus 1 HPIV-1, C35 8 ATCC Human parainfluenza virus 1 HPIV-1, C35 9 ATCC Human parainfluenza virus 2 (HPIV-2), Greer 10 ATCC Human parainfluenza virus 3 HPIV-1, C243 11 ATCC Streptococcus pneumoniae 12 ATCC Mycoplasma pneumoniae 13 VR-1435 Chlamydophila pneumoniae strain J-21 14 ATCC Legionella pneumophila subsp pneumophila 15 ATCC Moraxella (Branhamella) catarrhalis 17 ATCC Bordetella pertussis 18 ATCC Bordetella pertussis 19 ATCC Proteus mirabilis 20 ATCC Aggregatibacter aphrophilus 21 ATCC Haemophilus haemolyticus 22 zeptometrix Pseudomonas aeruginosa 23 zeptometrix Acinetobacter baumannii 24 ATCC Fluoribacter bozemanae (Legionella bozemanae) 25 ATCC Legionella nisa 26 zeptometrix Streptococcus pyogenes 27 zeptometrix Streptococcus pyogenes 28 zeptometrix Streptococcus mitis 30 zeptometrix Streptococcus mitis 31 zeptometrix Streptococcus bovis 31 zeptometrix Streptococcus bovis 31 zeptometrix Streptococcus anginosus 32 zeptometrix Bordetella bronchiseptica 33 zeptometrix Bordetella bronchiseptica 34 HPKTCC Neisseria meningitides 35 zeptometrix Klebsiella pneumoniae 36 vircell Chlamydia trachomatis	3	ATCC		
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31 zeptometrix Streptococcus anginosus 32 zeptometrix Bordetella bronchiseptica 33 zeptometrix Bordetella holmesii 34 HPKTCC Neisseria meningitides 35 zeptometrix Klebsiella pneumoniae 36 vircell Chlamydia trachomatis	29	zeptometrix	Streptococcus mitis	
32 zeptometrix Bordetella bronchiseptica 33 zeptometrix Bordetella holmesii 34 HPKTCC Neisseria meningitides 35 zeptometrix Klebsiella pneumoniae 36 vircell Chlamydia trachomatis	30	zeptometrix	Streptococcus bovis	
33 zeptometrix Bordetella holmesii  34 HPKTCC Neisseria meningitides  35 zeptometrix Klebsiella pneumoniae  36 vircell Chlamydia trachomatis	31	zeptometrix	Streptococcus anginosus	
34 HPKTCC Neisseria meningitides 35 zeptometrix Klebsiella pneumoniae 36 vircell Chlamydia trachomatis	32	zeptometrix	Bordetella bronchiseptica	
35 zeptometrix Klebsiella pneumoniae 36 vircell Chlamydia trachomatis	33	zeptometrix	Bordetella holmesii	
36 vircell Chlamydia trachomatis	34	HPKTCC	Neisseria meningitides	
<u> </u>	35	zeptometrix Klebsiella pneumoniae		
37 vircell Neisseria gonorrhea	36	vircell	Chlamydia trachomatis	
	37	vircell	Neisseria gonorrhea	

38	ATCC	Trichononas Vaginalis	
39	ATCC	Mycoplasma hominis	
40	ATCC	Mycoplasma genitalium	
41	ATCC	Ureaplasma urealyticum	
42	ATCC	Ureaplasma parvum	
43	ATCC	Gardnerella vaginalis	
44	ATCC	Haemophilus ducreyi	
45	ATCC	Candida albicans	
46	ATCC	Lactobacillus acidophilus	
47	ATCC	Escherichia coli	
48	ATCC	Bacteroides fragilis	
49	ATCC	Enterobacter cloacae	

#### 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 B Detection Kit, Repeatability test was performed two(2) runs per day, three(3) replicates per run, during consecutive twenty(20) 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 B 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

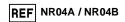
The clinical performance study was performed in the clinical laboratory with the specimen collected from various sources, such a hospitals or clinics. The comparable CE-marked product already available on EU market was used as reference test.

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
229E	NPS	98.52% [95% CI: 94.75-99.82]	100% [95% CI: 99.89-100]
OC43	NPS	98.89% [95% CI: 96.04-99.87]	100% [95% CI: 99.88-100]







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

14. SYMBOLS

NL63	NPS	99.13% 100% [95% CI: 95.25-99.98] [95% CI: 99.89-100]	
HEV	NPS	97.52% 99.97% [95% CI: 92.93-99.49] [95% CI: 99.83-100.0	
HRV	NPS	97.21% [95% CI: 94.78-98.72]	99.80% [95% CI: 99.57-99.93]
BoV	NPS	96.35% 99.84% [95% CI: 92.63-98.52] [95% CI: 99.63-9	
HKU1	NPS	100% 100% [95% CI: 96.31-100] [95% CI: 99.89-1	
PIV4	NPS	100% [95% CI: 96.23-100]	100 % [95% CI: 99.89-100]
MPV	NPS	99.35% [95% CI: 96.41-99.98]	100% [95% CI: 99.89-100]
229E	NPS	98.52% [95% CI: 94.75-99.82]	100% [95% CI: 99.89-100]

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

14. 31WBUL3				
REF	LOT	M	$\subseteq$	
Catalogue number	Batch code	Date of manufacture	Use-by date	Distributor
IVD	1	$\triangle$	[]i	
In vitro diagnostic medical device	Upper limit of temperature	Caution	Consult instruction for use	Importer
***	∑ ⟨n>	EC REP	€	UDI
Manufacturer	Contains sufficient for <n> tests</n>	Authorized representative in the European Community	Conformity to European Directive 98/79/EC	Unique Device Identification



GeneMatrix Inc.

Manufacturing site
7F, #8, Korea Bio Park, 700, Daewangpangyo-ro,
Bundang-gu, Seongnam-si, Gyeonggi-do, 13488
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Issue date: 2022 07

6



REF NR04A / NR04B



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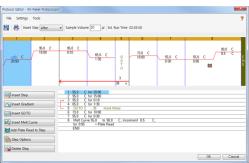
#### **Appendix. PCR Instrument Operation**

#### CFX96<sup>™</sup> Dx System (Bio-Rad)

#### 1. Protocol Setup

- 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  $\mu$ L.

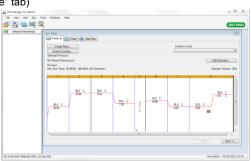




Segment	Tm(°C)	Time	Cycles
1	55	20 min	1
2	95	15 min	1
3	95	10 sec	40
4	65	90 sec	40
5	73	10 min	1
6	55	30 sec	1
7*		curve 55 °C ~ 90 °C (5	

<sup>\*</sup> 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.



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

Sample Type
'Unknown' : Clinical samples
'Negative control'
'Positive control'

③ Click the check box for the fluorescent substance (FAM, HEX, Cal Red 610, Quasar 670) of the selected well.





REF NR04A / NR04B



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

④ Click the "Settings" to set the plate type. (Settings -> Plate Type -> BR white)



- S Click the "OK" and save a new Plate set-up file.
- 6 The Experimental Setup screen opens and checks the set plate. Click the "Next" (Or click the "Start Run" tab)



#### 3. Start Run

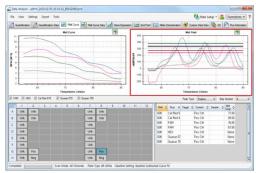
In the Experiment Setup Start Run Tab screen, click the "Close Lid" to close the lid of the equipment. (If the lid is closed, skip the step)

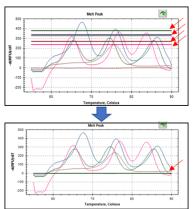


- ② Click the "Start Run".
- 3 The operating file is stored in the user's designated folder, and the equipment begins to operate.

#### 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".





8