Genematrix

INSTRUCTIONS FOR USE

NeoPlex™ HPV29 Detection

REF

NS02A / NS02B

GENEMATRIX INC.

CE IVD

Table of contents

Genematrix

REF NS02A / NS02B

NeoPlex™ HPV29 Detection

CE IVD

Multiplex Real-time PCR Reagents for Human papilloma virus detection
For professional *in vitro* diagnostic use only

1. INTENDED USE

The 'NeoPlex™ HPV29 Detection' is a qualitative *in vitro* test for the simultaneous detection and confirmation of cervical cancer-causing pathogens HPV(human papillomavirus) 29 genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 6, 11, 26, 40, 42, 43, 44, 53, 54, 61, 67, 69, 70, 73, 82) from liquid-based cytology specimen. This test kit is intended for professional use.

2. KIT CONTENTS

The 'NeoPlex™ HPV29 Detection' components are shown in the table below.

1) NS02A (96 Tests)

Contents	Volume(96T)	Storage condition	Shelf life
4X NeoPlex PCR Master Mix	500 µL x 2 Vial	Upper limit - 20 ℃	12 months (Before opening) 6 months (After opening)
HPV29 PPM 1	500 µL x 1 Vial		
HPV29 PPM 2	500 µL x 1 Vial		
HPV29 Positive Control(PC) 1	100 µL x 1 Vial		
HPV29 Positive Control(PC) 2	100 µL x 1 Vial		
HPV29 Internal Control(IC)	1 mL x 1 Vial		
DW(DNase-free Water)	1.5 mL x 1 Vial		

2) NS02B (50 Tests)

Contents	Volume(50T)	Storage condition	Shelf life
4X NeoPlex PCR Master Mix	250 µL x 2 Vial	Upper limit - 20 ℃	12 months (Before opening) 6 months (After opening)
HPV29 PPM 1	250 µL x 1 Vial		
HPV29 PPM 2	250 µL x 1 Vial		
HPV29 Positive Control(PC) 1	50 µL x 1 Vial		
HPV29 Positive Control(PC) 2	50 µL x 1 Vial		
HPV29 Internal Control(IC)	0.5 mL x 1 Vial		
DW(DNase-free Water)	0.75 mL x 1 Vial		

3. COMPATIBLE INSTRUMENT

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

4. NUCLEIC ACID EXTRACTION

Manufacturer	Instrument (Cat No.)	Extraction Kit (Cat No.)
Qiagen	N/A (Manual)	QIAamp DSP DNA Blood Mini kit (61104)
Roche Life Science	Roche MagNA Pure 96 system (06 541 089 001)	DNA and Viral NA Small Volume Kit (06543588001)
Hanwool TPC	NC-15 PLUS (HWTD-01-48)	AlphaPrep™ Viral DNA/RNA Extraction Kit (VDR-B096V)

5. ADDITIONAL REQUIRED EQUIPMENT & MATERIALS

- 0.2 mL 8-Tube PCR Strips without Caps, low profile, white (Bio-Rad, Inc., Cat No. TLS0851)
- Optical Flat 8-Cap Strips for PCR Tubes (Bio-Rad, Inc., Cat No. TCS0803)
- Pipettes set, P2/P10, P20, P200, and P1000 aerosol barrier tips
- Micro Centrifuge
- Vortex mixer

6. KIT STORAGE AND STABILITY

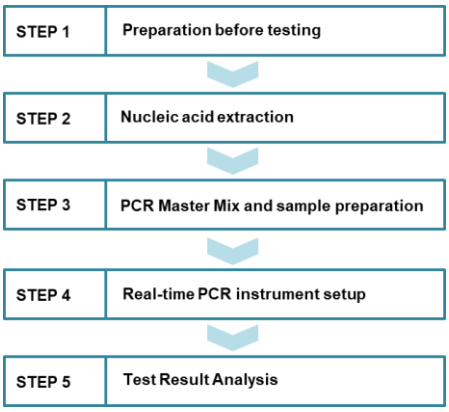
- Disposable powder-free gloves
- Store the kit below -20℃.
- Kit materials are stable until the expiration date printed on the label under un-opened condition.
- Kit's shelf life is twelve(12) months.
- Please use the reagents within six(6) months after opening.

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™ HPV29 Detection and/or specimens.
3. Do not smoke, drink, or eat while handling NeoPlex™ HPV29 Detection 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).
8. The use of filter tips is recommended to prevent contamination of the product.
9. 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.
10. Use the sterilized consumable laboratory supplies. Do not reuse it.
11. 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.
12. Before using, read this instruction for use carefully.
13. Use calibrated measuring tools. (e.g. pipette)
14. Please check the expiration date before using the reagent.

15. Keep Positive Control(PC) separately when using to avoid contamination.
16. Before starting the PCR, make sure the lid is closed properly.
17. Dispose the product in accordance with local or national regulations.
18. 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 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: Liquid based cytology specimen.
B. Store specimens at 2~8 °C for no longer than 4 weeks. For pro-longed storage, Freeze under -20 °C condition.
C. Extracted nucleic acids should be stored at -20 °C or lower.
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.
- 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, extract nucleic acid from the sample. Nucleic acid extraction can be done by automated purification system or using manual prep kits.

We recommend following the table at the bottom nucleic acid extraction kit/automatic machine for nucleic acid extraction. Also, for nucleic acid extraction, follow the manufacturer's protocol.

Instrument (Cat No.)	Reagent (Cat No.)	manufacturer	Elution volume	Sample volume
N/A (Manual)	QIAamp DSP DNA Blood Mini Kit	Qiagen	80 µL	200 µL
MagNA Pure 96	MagNA Pure 96 DNA and Viral Small Volume Kit	Roche Life Science	50 µL	200 µL
NC-15 PLUS (HWTD-01-48)	AlphaPrep™ Viral DNA/RNA Extraction Kit (VDR-B096V)	Hanwool TPC	50 µL	200 µL

STEP 3. PCR Master Mix and sample preparation

1) Prepare the Master Mix

① PCR Mixture 1

Contents	Volume (1 test)
4X NeoPlex PCR Master Mix	5 µL
HPV29 PPM 1	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 PCR Mixture 2

Contents	Volume (1 test)
4X NeoPlex PCR Master Mix	5 µL
HPV29 PPM 2	5 µL
DW(DNase-free Water)	5 µL
Total Volume	15 µL

2) Mix by inversion 5 times or by vortexing and centrifuging briefly.
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	Volume (1 test)
PCR Mixture 1 or PCR Mixture 2	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 HPV29 Positive Control(PC) 1 or HPV29 Positive Control(PC) 2 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(PC).
- 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) Selection of fluorescence channels.

<HPV PPM 1>		<HPV PPM 2>	
Target	**Dye**	**Target**	**Dye**
HPV 33	FAM	HPV 82	FAM
HPV 16	FAM	HPV 6	FAM
HPV 35	FAM	HPV 53	FAM
HPV 18	HEX	HPV 61	HEX
HPV 66	HEX	HPV 70	HEX
HPV 68	Cal Red 610	HPV 73	HEX
HPV 58	Cal Red 610	HPV 26	Cal Red 610
HPV 31	Cal Red 610	HPV 67	Cal Red 610
HPV 39	Quasar 670	HPV 40	Cal Red 610
HPV 56	Quasar 670	HPV 44	Quasar 670
HPV 52	Quasar 670	HPV 11	Quasar 670
HPV 45	Quasar 705	HPV 42	Quasar 670
HPV 59	Quasar 705	HPV 54	Quasar 705
HPV 51	Quasar 705	HPV 43	Quasar 705
IC	HEX	HPV 69	Quasar 705
		IC	HEX

2) 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	30 sec	40
4	65	75 sec	
5	73	10 min	1
6	55	30 sec	1
7*	Melting curve 55 ℃ ~ 91 ℃ (5s/0.5℃)		

** Segment 7: Melting curve measurement*

STEP 5. Test result analysis

For the analysis of the test result after PCR amplification, take the RFU value result and interpret the according to '9. INTERPRETATION OF TEST RESULTS'.

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

HPV PPM 1			
Target	**Dye**	**Melt Tm**	**Cut-off(RFU*)**
HPV 33	FAM	65.0 ± 1.5℃	≥ 93.5
HPV 16	FAM	73.0 ± 1.5℃	≥ 98.3
HPV 35	FAM	80.5 ± 1.5℃	≥ 93.9
HPV 18	HEX	75.0 ± 1.5℃	≥ 95.1
HPV 66	HEX	83.5 ± 1.5℃	≥ 98.2
HPV 68	Cal Red 610	64.0 ± 1.5℃	≥ 93.0
HPV 58	Cal Red 610	71.5 ± 1.5℃	≥ 91.5
HPV 31	Cal Red 610	77.5 ± 1.5℃	≥ 94.5
HPV 39	Quasar 670	64.5 ± 1.5℃	≥ 99.7
HPV 56	Quasar 670	73.0 ± 1.5℃	≥ 97.8
HPV 52	Quasar 670	79.0 ± 1.5℃	≥ 97.3
HPV 45	Quasar 705	66.0 ± 1.5℃	≥ 92.0
HPV 59	Quasar 705	73.5 ± 1.5℃	≥ 95.1
HPV 51	Quasar 705	80.5 ± 1.5℃	≥ 98.7
IC**	HEX	66.0 ± 1.5℃	≥ 100

HPV PPM 2			
Target	**Dye**	**Melt Tm**	**Cut-off(RFU*)**
HPV 82	FAM	65.5 ± 1.5℃	≥ 99.4
HPV 6	FAM	73.5 ± 1.5℃	≥ 97.6
HPV 53	FAM	82.5 ± 1.5℃	≥ 96.6
HPV 61	HEX	64.5 ± 1.5℃	≥ 95.8
HPV 70	HEX	73.0 ± 1.5℃	≥ 95.4
HPV 73	HEX	78.0 ± 1.5℃	≥ 96.0
HPV 26	Cal Red 610	66.0 ± 1.5℃	≥ 91.5
HPV 67	Cal Red 610	73.0 ± 1.5℃	≥ 94.3
HPV 40	Cal Red 610	81.5 ± 1.5℃	≥ 97.7
HPV 44	Quasar 670	64.0 ± 1.5℃	≥ 97.4
HPV 11	Quasar 670	70.5 ± 1.5℃	≥ 97.9
HPV 42	Quasar 670	79.5 ± 1.5℃	≥ 99.0
HPV 54	Quasar 705	67.5 ± 1.5℃	≥ 98.4
HPV 43	Quasar 705	76.0 ± 1.5℃	≥ 99.5
HPV 69	Quasar 705	84.5 ± 1.5℃	≥ 95.0
IC**	HEX	86.5 ± 1.5℃	≥ 100

* RFU(–d(RFU)/dT): Relative fluorescence units
** IC: Internal Control(IC) is an IC signal that can monitor the entire process from specimen preparation to interpretation of results.

2. Interpretation of result

Target	IC	Result	
+	+	Detected	- Target is detected.

-	+	Not detected	- Target is not detected.
-	-	Not detected (IC 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. - If the IC is not detected after the retest, To verify the nucleic acid extraction process and the presence of PCR inhibitors, the experimental process can be verified by adding 10μL of HPV29 Internal Control (IC) contained in the product to the sample and then extracting nucleic acid.
+	-	Detected	- If the nucleic acid concentration id high in the sample, IC signal may be attenuated. - If a target pathogen is detected in the sample and no IC is detected, the target pathogen is determined to be detected. - if you want to check IC, dilute the template nucleic acid in distilled water and repeat the PCR with the diluted nucleic acid.

* IC is not necessary for the interpretation of positive or negative results and high load of pathogen's nucleic acid results in the low signal or negative signal of IC.
* If PC are identified above its cut-off, it may indicate that the kit has been handled in an improper way and should be replaced with a new one.

3. Application examples of clinical samples

No	Target																Result
	33	16	35	18	66	68	58	31	39	56	52	45	59	51	IC	
Sample 1	-	+	-	-	-	+	-	-	-	-	-	+	-	-	+	HPV 16, 68, 45
Sample 2	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	HPV 18, 39
Sample 3	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	HPV 33
Sample 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	Not detected
Sample 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Not detected (IC Invalid)
Positive Control 1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Positive Control
Negative Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Negative Control

No	Target																Result
	82	6	53	61	70	73	26	67	40	44	11	42	54	43	69	IC	
Sample 1	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	HPV 6
Sample 2	-	-	+	-	+	-	-	-	-	-	-	-	-	+	-	+	HPV 53, 70, 43
Sample 3	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	HPV 82, 42
Sample 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	Not detected
Sample 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Not detected (IC Invalid)
Positive Control 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Positive Control
Negative Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Negative Control

4. Precaution for result analysis

1) If the Positive Control(HPV29 Positive Control(PC) 1, HPV29 Positive Control(PC) 2) and RFU(-d(RFU)/dT) value is outside the acceptable range, invalidate all relevant tests and retested.

2) If amplification is observed in the Negative Control(NC), it is determined that contamination occurred during the experiment and a retest is conducted. However, if the same result is confirmed after the retest, it is recommended to remove the source of contamination and perform the nucleic acid extraction process again.

3) Internal Control(IC) should always be amplified, and if the concentration of nucleic acid in the sample is high, the IC signal may be impaired, resulting in a reduction or offset of the signal. If you want to check the IC, it is recommended to dilute the sample and retest it.

4) If both the target and IC are determined to be negative, the presence of inhibitors within the extracted nucleic acid hindered the PCR reaction. It is recommended that the nucleic acid extraction process be carried out again.

10. QUALITY CONTROL

NeoPlex™ HPV29 Detection includes HPV29 Positive Control(PC) 1, HPV29 Positive Control(PC) 2 as Positive Control and DW(DNase-free Water) as Negative Control(NC). For all runs, valid test results must be obtained for PC and NC. Positive Control(PC) result must be Positive (Valid). Negative Control(NC) result must be Negative (Valid). If the Positive and Negative Control(NC) results are consistently invalid, contact us for technical assistance.

11. TROUBLE SHOOTING

1. If all signals are not detected.

Potential causes	Solution
Error in specimen collection	- If target and IC, PC 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.
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.
Using frozen/thawed over four(4) times reagent	- Do not freeze and thaw repeatedly exceed four(4) times. Because sensitivity of the reagent may decrease. - Check the Freezing/thawing times 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 the Negative Control signal is detected/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 the Positive Control signals is Negative/False negative.

Potential causes	Solution
Error in specimen collection	- 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.
If PC signal is invalid	- If PC is identified above the cut-off, it may indicate that the kit has been handled in improper way should be replaced with a new one.

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.

No	Target	LoD	No	Target	LoD
1	HPV33	0.51 copies/ul	16	HPV6	1.24 copies/ul
2	HPV16	0.46 copies/ul	17	HPV53	0.52 copies/ul
3	HPV35	1.30 copies/ul	18	HPV61	1.40 copies/ul
4	HPV18	0.59 copies/ul	19	HPV70	0.55 copies/ul
5	HPV66	1.04 copies/ul	20	HPV73	1.08 copies/ul
6	HPV68	1.12 copies/ul	21	HPV26	0.98 copies/ul
7	HPV58	1.68 copies/ul	22	HPV67	1.24 copies/ul
8	HPV31	1.72 copies/ul	23	HPV40	0.91 copies/ul
9	HPV39	0.53 copies/ul	24	HPV44	2.11 copies/ul
10	HPV56	1.94 copies/ul	25	HPV11	2.22 copies/ul
11	HPV52	1.50 copies/ul	26	HPV42	3.10 copies/ul
12	HPV45	0.56 copies/ul	27	HPV54	0.83 copies/ul
13	HPV59	0.55 copies/ul	28	HPV43	1.97 copies/ul
14	HPV51	0.51 copies/ul	29	HPV69	1.16 copies/ul
15	HPV82	1.35 copies/ul			

1.2 Cut-off value

For the cut-off establishment, RFU value was set up as shown below in the table for each target.

HPV PPM 1			
Target	**Dye**	**Melt Tm**	**Cut-off(RFU*)**
HPV 33	FAM	65.0 ± 1.5℃	≥ 93.5
HPV 16	FAM	73.0 ± 1.5℃	≥ 98.3
HPV 35	FAM	80.5 ± 1.5℃	≥ 93.9
HPV 18	HEX	75.0 ± 1.5℃	≥ 95.1
HPV 66	HEX	83.5 ± 1.5℃	≥ 98.2
HPV 68	Cal Red 610	64.0 ± 1.5℃	≥ 93.0
HPV 58	Cal Red 610	71.5 ± 1.5℃	≥ 91.5
HPV 31	Cal Red 610	77.5 ± 1.5℃	≥ 94.5
HPV 39	Quasar 670	64.5 ± 1.5℃	≥ 99.7
HPV 56	Quasar 670	73.0 ± 1.5℃	≥ 97.8
HPV 52	Quasar 670	79.0 ± 1.5℃	≥ 97.3
HPV 45	Quasar 705	66.0 ± 1.5℃	≥ 92.0
HPV 59	Quasar 705	73.5 ± 1.5℃	≥ 95.1
HPV 51	Quasar 705	80.5 ± 1.5℃	≥ 98.7
IC**	HEX	66.0 ± 1.5℃	≥ 100

HPV PPM 2			
Target	**Dye**	**Melt Tm**	**Cut-off(RFU*)**
HPV 82	FAM	65.5 ± 1.5℃	≥ 99.4
HPV 6	FAM	73.5 ± 1.5℃	≥ 97.6
HPV 53	FAM	82.5 ± 1.5℃	≥ 96.6
HPV 61	HEX	64.5 ± 1.5℃	≥ 95.8
HPV 70	HEX	73.0 ± 1.5℃	≥ 95.4
HPV 73	HEX	78.0 ± 1.5℃	≥ 96.0
HPV 26	Cal Red 610	66.0 ± 1.5℃	≥ 91.5
HPV 67	Cal Red 610	73.0 ± 1.5℃	≥ 94.3
HPV 40	Cal Red 610	81.5 ± 1.5℃	≥ 97.7
HPV 44	Quasar 670	64.0 ± 1.5℃	≥ 97.4
HPV 11	Quasar 670	70.5 ± 1.5℃	≥ 97.9
HPV 42	Quasar 670	79.5 ± 1.5℃	≥ 99.0
HPV 54	Quasar 705	67.5 ± 1.5℃	≥ 98.4
HPV 43	Quasar 705	76.0 ± 1.5℃	≥ 99.5
HPV 69	Quasar 705	84.5 ± 1.5℃	≥ 95.0
IC**	HEX	86.5 ± 1.5℃	≥ 100

* RFU(–d(RFU)/dT): Relative fluorescence units
** IC: Internal Control(IC) is an IC signal that can monitor the entire process from specimen preparation to interpretation of results.

2. Analytical Specificity

2.1 Interference

Total eight(8) substances, endogenous and exogenous source, were studied to determine their interfering effect and no interference reactions was found with the concentration as below.

No	Types	Interfering substance	Concentration
1	Endogenous substances	Blood plasma	5 %
2		Red blood cell	5 %
3		Buffy coat	5 %
4		Human genomic DNA	1 ug
5	Exogenous substances	PBS	1 %
6		Douches	2 %
			5 %
7		Antifungal ointment	2 %
			5 %
8		Vaginal lubricants	2 %
			5 %

2.2 Cross reactivity

For analytical specificity, three(3) times of cross reactivity study using twenty-one`(21) different pathogens were performed to determine the analytical specificity. As a result, PCR amplification and cross reactivity were not observed with the below pathogens.

No	Strain No.	Strain	Concentration
1	ATCC 49145D-5	Gardnerella vaginalis	$1.11X10^7$ copies/ul
2	ATCC 700724D-5	Haemophilus ducreyi	$1.09X10^7$ copies/ul
3	ATCC 10231D-5	Candida albicans	$1.16X10^6$ copies/ul
4	ATCC VR-539	Human herpesvirus 1 DNA	$1X10^6$ copies/ul
5	ATCC VR-540	Human herpesvirus 2 DNA	$1X10^6$ copies/ul
6	ATCC 4357D-5	Lactobacillus acidophilus	$7.89X10^8$ copies/ul
7	ATCC 700669D-5	Streptococcus pneumoniae	$4.3X10^8$ copies/ul
8	ATCC 700928D-5	Escherichia coli	$7.14X10^7$ copies/ul
9	ATCC 25285D-5	Bacteroides fragilis	$8.4X10^7$ copies/ul
10	ATCC 13047D-5	Enterobacter cloacae	$6.09X10^7$ copies/ul
11	ATCC 12453D	Proteus mirabilis	$1.14X10^8$ copies/ul
12	ATCC 700802D-5	Enterococcus faecalis	$1.32X10^8$ copies/ul
13	ATCC 12228D-5	Staphylococcus epidermidis	$1.41X10^8$ copies/ul
14	HPKTCC B3204	Neisseria meningitidis	$1x10^5$ copies/ul
15	ATCC VR-348BD	Chlamydia trachomatis(CT)	$8.89X10^5$ copies/ul
16	ATCC 53420D-5	Neisseria gonorrhoeae(NG)	$2.15X10^7$ copies/ul
17	ATCC 23114D	Mycoplasma hominis(MH)	$1.39X10^4$ copies/ul
18	ATCC 33530D	Mycoplasma genitalium(MG)	$1.6X10^4$ copies/ul
19	ATCC 30001D	Trichomonas vaginalis(TV)	$4.3X10^5$ copies/ul
20	ATCC 33695	Ureaplasma urealyticum(UU)	$1.06X10^4$ copies/ul
21	ATCC 27815	Ureaplasma parvum(UP)	10^8 ccu/vial

The result no cross reactivity was found when testing with the target genotype of PPM 1 or PPM 2 was crossed.

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 (174/174) (95% CI: 97.90%-100%) for each negative specimen were determined, respectively.

3. Precision

3.1 Repeatability

To evaluate the repeatability of NeoPlex™ HPV29 Detection, 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™ HPV29 Detection is acceptable.

Contents	Agreement	95% CI
Within-run	100 %	99.97-100
Between-run	100 %	99.90-100
Between-Day	100 %	99.79-100

3.2 Reproducibility

To evaluate the reproducibility of NeoPlex™ HPV29 Detection, Reproducibility tests were performed two(2) runs per day, three(3) replicates per run, during consecutive five(5) days. Four(4) variations, Lot/Tester/Instrument/Site, were considered for each test. Samples were tested using high, medium, low concentrations of positive samples and negative control. For each test, we confirmed that every test results are met the acceptance criteria: within 10% of CV, 100% agreement and the reproducibility (Lot, Tester, Instrument, Site) of NeoPlex™ HPV29 Detection is acceptable.

4. Clinical Evaluation

To claim the intended use, “The 'NeoPlex™ HPV29 Detection’ is a qualitative in vitro test for the simultaneous detection and confirmation of cervical cancer-causing pathogens HPV(human papillomavirus) 29 genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 6, 11, 26, 40, 42, 43, 44, 53, 54, 61, 67, 69, 70, 73, 82) from liquid-based cytology specimen. This test kit is intended for professional use.”, the clinical performance evaluation was performed.

4.1 Clinical Accuracy (Clinical Sensitivity & Specificity)

We concluded that the clinical sensitivity and specificity of the test reagent is valid to meet the clinical effectiveness.

Target	Clinical sensitivity	Clinical specificity
HPV 33	98.02% [95% CI: 93.03 - 99.76]	99.84% [95% CI: 99.54-99.97]
HPV 16	99.54% [95% CI: 97.47 - 99.99]	99.89% [95% CI: 99.59 - 99.99]
HPV 35	98.90% [95% CI: 94.03 - 99.97]	99.79% [95% CI: 99.46 - 99.94]
HPV 18	99.19% [95% CI: 95.59 - 99.98]	100.00% [95% CI: 99.80 - 100.00]
HPV 66	98.32% [95% CI: 94.06 - 99.80]	99.89% [95% CI: 99.61 - 99.99]
HPV 68	100.00% [95% CI: 96.58 - 100.00]	99.84% [95% CI: 99.53 - 99.97]
HPV 58	98.80% [95% CI: 95.74 - 99.85]	99.95% [95% CI: 99.69 - 100.00]
HPV 31	100.00% [95% CI: 96.61 - 100.00]	99.89% [95% CI: 99.62 - 99.99]
HPV 39	99.35% [95% CI: 96.41 - 99.98]	99.89% [95% CI: 99.61 - 99.99]
HPV 56	98.90% [95% CI: 94.03 - 99.97]	99.84% [95% CI: 99.54 - 99.97]
HPV 52	100.00% [95% CI: 98.09 - 100.00]	99.89% [95% CI: 99.60 - 99.99]
HPV 45	100.00% [95% CI: 95.32 - 100.00]	100.00% [95% CI: 99.81 - 100.00]
HPV 59	100.00% [95% CI: 95.70 - 100.00]	99.95% [95% CI: 99.71 - 100.00]
HPV 51	98.99% [95% CI: 94.50 - 99.97]	99.95% [95% CI: 99.71 - 100.00]
HPV 82	98.63% [95% CI: 92.60 - 99.97]	99.90% [95% CI: 99.62 - 99.99]
HPV 6	100.00% [95% CI: 95.98 - 100.00]	100.00% [95% CI: 99.81 - 100.00]
HPV 53	99.37% [95% CI: 96.52 - 99.98]	99.78% [95% CI: 99.44 - 99.94]
HPV 61	100.00% [95% CI: 95.26 - 100.00]	99.90% [95% CI: 99.62 - 99.99]
HPV 70	98.72% [95% CI: 93.06 - 99.97]	99.95% [95% CI: 99.71 - 100.00]

HPV 73	98.44% [95% CI: 91.60% - 99.96]	100.00% [95% CI: 99.81 - 100.00]
HPV 26	100.00% [95% CI: 96.45 - 100.00]	100.00% [95% CI: 99.80 - 100.00]
HPV 67	100.00% [95% CI: 96.15 - 100.00]	99.79% [95% CI: 99.46 - 99.94]
HPV 40	98.95% [95% CI: 94.27 - 99.97]	99.89% [95% CI: 99.62 - 99.99]
HPV 44	98.75% [95% CI: 93.23 - 99.97]	99.74% [95% CI: 99.39 - 99.91]
HPV 11	100.00% [95% CI: 96.23 - 100.00]	100.00% [95% CI: 99.81 - 100.00]
HPV 42	98.37% [95% CI: 94.25 - 99.80]	99.73% [95% CI: 99.37 - 99.91]
HPV 54	100.00% [95% CI: 96.64 - 100.00]	99.89% [95% CI: 99.62 - 99.99]
HPV 43	100.00% [95% CI: 95.65 - 100.00]	99.95% [95% CI: 99.71 - 100.00]
HPV 69	99.21% [95% CI: 95.66 - 99.98]	99.84% [95% CI: 99.53 - 99.97]

4.2 The agreement between NeoPlex™ HPV29 Detection and the comparator (Correlation)

Overall agreement between the NeoPlex™ HPV29 Detection and comparator method was confirmed as below.

Target	Positive agreement	Negative agreement	Total agreement	Kappa value
HPV33	99.01% [95% CI: 94.61-99.97]	99.89% [95% CI: 99.62-99.99]	99.85% [95% CI: 99.56-99.97]	0.984
HPV16	100.00% [95% CI: 98.32-100.00]	99.94% [95% CI: 99.69-100.00]	99.95% [95% CI: 99.72-100.00]	0.997
HPV35	98.91% [95% CI: 94.09-99.97]	99.84% [95% CI: 99.54-99.97]	99.80% [95% CI: 99.49-99.95]	0.977
HPV18	100.00% [95% CI: 97.02-100.00]	99.95% [95% CI: 99.70-100.00]	99.95% [95% CI: 99.72-100.00]	0.996
HPV66	99.16% [95% CI: 95.41-99.98]	99.95% [95% CI: 99.70-100.00]	99.90% [95% CI: 99.64-99.99]	0.991
HPV68	99.07% [95% CI: 94.95-99.98]	99.89% [95% CI: 99.62-99.99]	99.85% [95% CI: 99.56-99.97]	0.985
HPV58	100.00% [95% CI: 97.76-100.00]	99.84% [95% CI: 99.52-99.97]	99.85% [95% CI: 99.56-99.97]	0.990
HPV31	100.00% [95% CI: 96.55-100.00]	99.79% [95% CI: 99.46-99.94]	99.80% [95% CI: 99.49-99.95]	0.980
HPV39	98.71% [95% CI: 95.42-99.84]	99.95% [95% CI: 99.70-100.00]	99.85% [95% CI: 99.56-99.97]	0.989
HPV56	98.92% [95% CI: 94.15-99.97]	99.95% [95% CI: 99.71-100.00]	99.90% [95% CI: 99.64-99.99]	0.989
HPV52	100.00% [95% CI: 98.10-100.00]	99.94% [95% CI: 99.69-100.00]	99.95% [95% CI: 99.72-100.00]	0.997
HPV45	100.00% [95% CI: 95.32-100.00]	100.00% [95% CI: 99.81-100.00]	100.00% [95% CI: 99.81-100.00]	1.000
HPV59	100.00% [95% CI: 95.60-100.00]	99.84% [95% CI: 99.54-99.97]	99.85% [95% CI: 99.56-99.97]	0.981
HPV51	100.00% [95% CI: 96.27-100.00]	99.89% [95% CI: 99.62-99.99]	99.90% [95% CI: 99.64-99.99]	0.989
HPV82	98.61% [95% CI: 92.50-99.96]	99.84% [95% CI: 99.54-99.97]	99.80% [95% CI: 99.49-99.95]	0.972
HPV6	100.00% [95% CI: 95.89-100.00]	99.89% [95% CI: 99.62-99.99]	99.90% [95% CI: 99.64-99.99]	0.988
HPV53	99.38% [95% CI: 96.59-99.98]	99.95% [95% CI: 99.70-100.00]	99.90% [95% CI: 99.64-99.99]	0.993
HPV61	100.00% [95% CI: 95.14-100.00]	99.79% [95% CI: 99.47-99.94]	99.80% [95% CI: 99.49-99.95]	0.973
HPV70	98.73% [95% CI: 93.15-99.97]	100.00% [95% CI: 99.81-100.00]	99.95% [95% CI: 99.72-100.00]	0.993
HPV73	100.00% [95% CI: 94.22-100.00]	99.95% [95% CI: 99.71-100.00]	99.95% [95% CI: 99.72-100.00]	0.992
HPV26	100.00% [95% CI: 96.45-100.00]	100.00% [95% CI: 99.80-100.00]	100.00% [95% CI: 99.81-100.00]	1.000
HPV67	100.00% [95% CI: 96.23-100.00]	99.89% [95% CI: 99.62-99.99]	99.90% [95% CI: 99.64-99.99]	0.989
HPV40	98.96% [95% CI: 94.33-99.97]	99.95% [95% CI: 99.71-100.00]	99.90% [95% CI: 99.64-99.99]	0.989
HPV44	98.77% [95% CI: 93.31-99.97]	99.79% [95% CI: 99.46-99.94]	99.75% [95% CI: 99.41-99.92]	0.968
HPV11	100.00% [95% CI: 96.23-100.00]	100.00% [95% CI: 99.81-100.00]	100.00% [95% CI: 99.81-100.00]	1.000
HPV42	98.37% [95% CI: 94.25-99.80]	99.73% [95% CI: 99.37-99.91]	99.65% [95% CI: 99.28-99.86]	0.970
HPV54	100.00% [95% CI: 96.67-100.00]	99.95% [95% CI: 99.70-100.00]	99.95% [95% CI: 99.72-100.00]	0.995
HPV43	100.00% [95% CI: 95.60-100.00]	99.89% [95% CI: 99.62-99.99]	99.90% [95% CI: 99.64-99.99]	0.987
HPV69	99.22% [95% CI: 95.72-99.98]	99.95% [95% CI: 99.70-100.00]	99.90% [95% CI: 99.64-99.99]	0.992

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. HPV29 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

REF	LOT			
Catalogue number	Batch code	Date of manufacture	Use-by date	Distributor
IVD				
In vitro diagnostic medical device	Upper limit of temperature	Caution	Consult instruction for use	Importer
	Σ <n>	EC REP	CE	UDI
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
7F, #B, Korea Bio Park, 700, Daewangpangyo-ro, Bundang-gu, Seongnam-si, Gyeonggi-do, 13488 REPUBLIC OF KOREA
Tel: +82-31-628-2045 Fax: +82-31-628-2002

EC REP MT Promedt Consulting GmbH
Ernst-Heckel-Straße 7
66386 St. Ingbert, Germany
Tel: +49-6894-581020, Fax: +49-6894-581021

CE IVD

Issue date: 2022.07

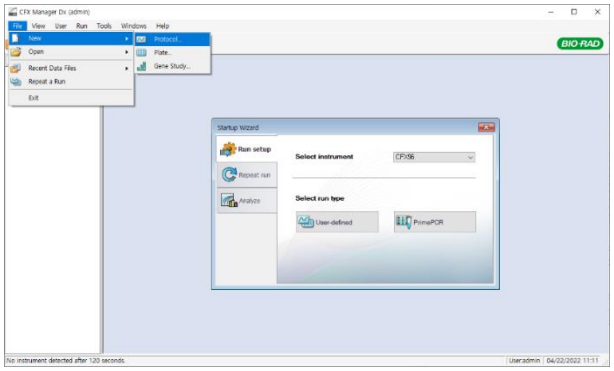
Appendix. PCR Instrument Operation

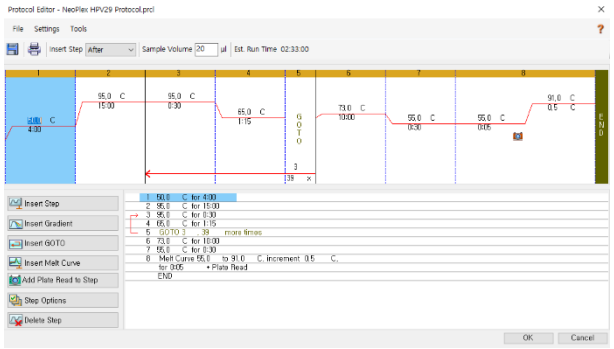
1) CFX96™ Dx System (Bio-Rad)

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.

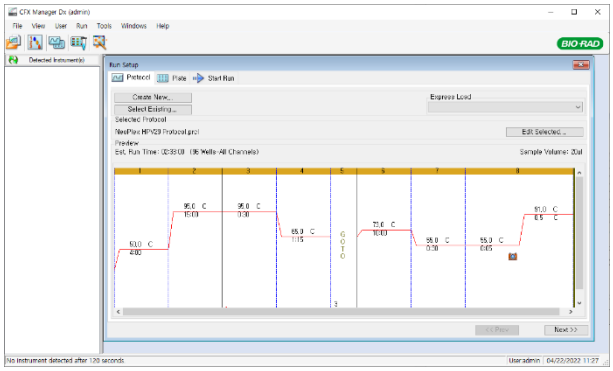




Segment	Tm(°C)	Time	Cycles
1	50	4 min	1
2	95	15 min	1
3	95	30 sec	40
4	65	75 sec	
5	73	10 min	1
6	55	30 sec	1
7*	Melting curve 55 ℃ ~ 91 ℃ (5s /0.5℃)		

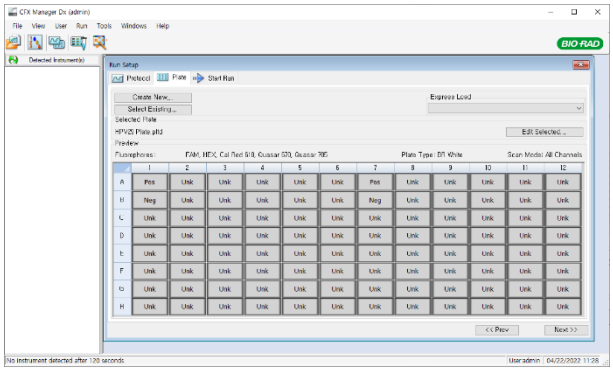
** Segment 7: Melting curve measurement*

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)

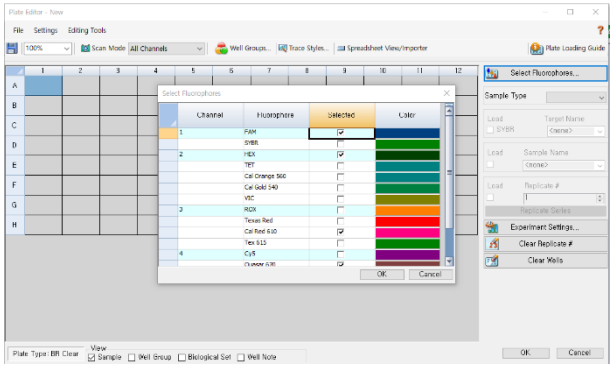


2. Plate Setup

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



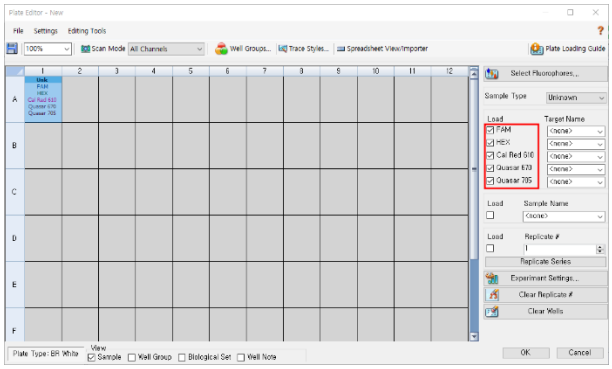
② Click the “Select Fluorophores”. Select the check box (FAM, HEX, Cal Red 610, Quasar 670, Quasar 705) for the fluorescent substance used for the experiment and click the OK button.



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

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

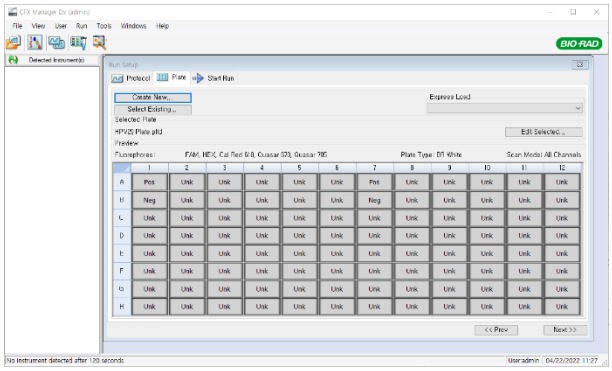
④ Click the check box for the fluorescent substance (FAM, HEX, Cal Red 610, Quasar 670, Quasar 705) of the selected well.



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)

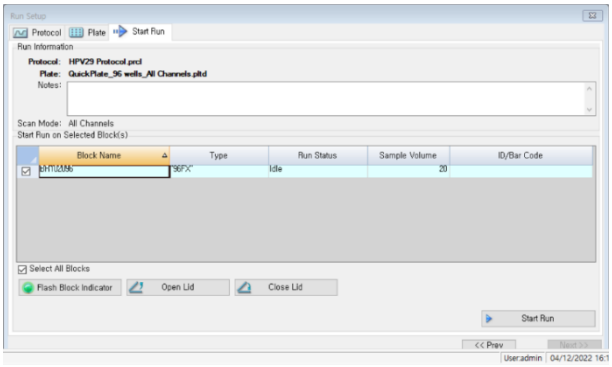


3. Start Run

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".